

Epidemiology of Plasmodium Parasitemia in General Population of Bannu District Khyber Pakhtunkhwa (Kp), Pakistan

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ABSTRACT

A parasitological survey was conducted to know the epidemiology of malaria in general population of Bannu district.Total four hundred and forty (440) blood samples were randomly collected in different localities of district Bannu from both males and females population of varying age group (1- >50) from the months of September 2013 to June 2014 and subjected to both Microscopy and Rapid Diagnostic Test(RDT). Malaria was highly prevalent 131/440 (29.77 % microscopy) and 97/440 (22.04% RDT) in the studied area. *Plasmodiumvivax* was found to be more prevalent by both microscopy 116/131 (88.54%) andRapid Diagnostic Test(RDT) 61/97 (62.3 %). Males of varying age groups were highly susceptible to malaria infection 80/235 (34.04 % by microscopy) and 59/246 (23.98% by RDT_s) than females of varying age groups 51/205 (24.87 % by microscopy) and 38/194 (19.58% by RDT_s). Both diagnostic results showed

that highest cases were found in a sector A, while the lowest prevalence of malaria infection was found in a sector C. *Plasmodium* infections in Bannu district are largely attributed to *Plasmodium vivax* but *Plasmodium falciparum* and mixed species infections are also prevalent. In addition, microscopic show best and accurate diagnosis as compare to Rapid Diagnostic Test Kit (RDT).

Keywords: *Pasmodiumvivax*, *Pasmodium falciparum*, Epidemiology, Bannu district, Khyber Pakhtunkhwa, Rapid Diagnostic Test (RDT)

INTRODUCTION

Malaria is one of the global public health problems and imposes a major burden on health in under developed countries of world. Half of world population is at risk of malaria with an estimated 250 million clinical cases and nearly one million deaths were reported. Malaria is a leading cause of morbidity and mortality in the developing world, especially in the sub Saharan Africa where the transmission rate is highest and there it is considered as a major obstruction to economic development (Awan *et al.*, 2012a).

Malaria is a Vector-borne infectious disease caused by Protozoan parasite of the genus *Plasmodium* (Snow *et al.*, 2005). Malaria is transmitted via the bite of a female *Anopheles* mosquito, which occurs mainly between dusk and dawn (Carter and Mendis, 2002). The disease is caused by five plasmodia; *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium knowlesi* (Awan *et al.*, 2012a). Of the five human malarial parasite species, *P. vivax* is the most widespread and *P. falciparum* is the most fatal one (Mendis *et al.*, 2001). It is widespread in tropical and subtropical regions, there are approximately 515 million cases of malaria, killing between one and three million people, the majority of whom are young children (Snow *et al.*, 2005), but pregnant women are also equally vulnerable (Greenwood *et al.*, 2008).

Plasmodium vivax is the major cause of malaria morbidity outside Africa (Carlton *et al.*, 2008). Pakistan is endemic for both *P. vivax* and *P. falciparum* malaria and about ¼ million episodes of malaria infection occur each year (Asif, 2008; Yasinzai and Kakarsulemankhel, 2009). Malaria transmission in Pakistan is markedly seasonal and prone to epidemic outbreaks in particular geographical areas, especially the Khyber Pakhtunkhwa (KP), the Baluchistan province and the Sindh province (Ministry of Health, 2010). The prevailing extensive agricultural practices, an

expansive irrigation network, and the monsoon rains act together to promote a favorable environment for malaria transmission in many areas of Pakistan (WHO, 2010).

Plasmodium falciparum and *P. vivax* are the only prevalent species of malaria parasite in the country. *P. vivax* is the predominant one (>74%) throughout Pakistan, with maximum number being reported from Khyber Pakhtunkhwa (49%) and FATA regions (World Malaria Report, 2009). The results of recent parasite sero-prevalence survey conducted in 19 highly endemic districts shows the highest parasite prevalence rate in FATA (116 per 1000 population) followed by Baluchistan (47.9 per 1000 population) (Khattak *et al.*, 2013).

Microscopy has historically been the mainstay of the diagnosis of malaria. A clinical diagnosis of malaria currently depends on the visualization of parasites by light microscopy of Giemsa-stained thick and thin blood smears (Sachet *et al.*, 2002). In malaria endemic regions, RDTs are currently rolled out by national malaria control programs as a tool for parasite based diagnosis (Drakeley *et al.*, 2009). In non-endemic settings, where microscopic expertise is lacking due to low incidence, RDTs have been reported to perform accurately and even better as compared to microscopy (Palmer *et al.*, 2003; Stauffer *et al.*, 2009). Several molecular methods based on the amplification of DNA have been developed for the detection of malarial infections in humans, but only one of these, using five PCRs, can differentiate between the four species of *Plasmodium* (Rubio *et al.*, 2005). In malaria endemic areas where transmission of both *P. falciparum* and *P. vivax* occurs, nested PCR detection of malaria parasites can be a very useful complement to microscopical examination in order to obtain the real incidence of each species (Zakeri *et al.*, 2009). Many studies have demonstrated the greater sensitivity and specificity of PCR compared to thick blood films. The detection of low *P. vivax* and *P. falciparum* parasitaemia by PCR, at levels undetectable by microscopy (Sedigheh Zakeri *et al.*, 2010; Yasin Zai *et al.*, 2009). Keeping in view the above, the present study was designed to carry out the high prevalence status of malaria in district Bannu and evaluate the comparison of microscopy and rapid diagnostic test (RDT) for the effective diagnosis of malarial parasites.

MATERIAL AND METHODS

Ethical consideration and Study Area

The study was approved by the Bio-ethical Committee of Government Post Graduate College Bannu. The study was conducted in Bannu district which lies between 32 ° 43' - 33 ° 06' N; 70 ° 22' - 57' E of Khyber Pakhtunkhwa (KP) province, from September, 2013 to June, 2014.

Bannu district is bounded on the north east by Karak district ,on the east by Mianwalidistrict, in the south by the DeraIsmail Khan (D.I. Khan) division and the west by tribal area adjoining Bannu district, South Waziristan Agency and Tribal area adjoining D.I. Khan Division (Fig. 1). The total area of the division is 4,391 km² (1981 census report). This populous district (552 persons /Km²) is listed among the most malaria-afflicted areas in Pakistan (Khatoonet al., 2010). By 1998 census report the total population of district Bannu is 677350. Majority of the peoples are very poor, having economic status very low and there is lack of facilities regarding proper health and awareness about the diseases transmission (1998 Census). At present time by census report of 2017 the population of Bannu district is more than 1167892. Mean daily temperatures range between 10.8°C - 32.9°C. The area experiences two rainy seasons: in March and during the summer monsoon that occurs in July and August. Malaria transmission peaks following the monsoon season (Khatoonet al., 2010).

Bannu district is divided by Malaria Control program into three main sectors A, B and C. These sectors are subdivided into sub-sectors. Sectors A includes seven sub –sectors i.e. a₁- a₇ containing 59 index localities. Among these seven sub-sectors three i.e. a₁, a₂, a₃ comprise the F.R. areas having 19 localities.Sector B also includes seven sub-sectors i.e. b₁ – b₇ comprising 65 index localities, similarly the sector C includes eight sub-sectors i.e. C₁ – C₈ having 94 index localities. Similarly each of the index localities has been subdivided into several villages.

Climate

Climate of Bannu district is hot during summer and cold in winter. High temperatures are recorded in the months of June to August when mercury rises up to 39⁰C during July. The coldest season is December – February when maximum temperature drops to 18⁰C during February and minimum to 02⁰C during December. The mean rain- fall is usually 62mm, most of it falling during March - June.The humidity is high during December – February, when maximum humidity raises upto 84.19% during January and minimum of 27.06% during May (Agriculture Research Station SaraiNaurangLakki).

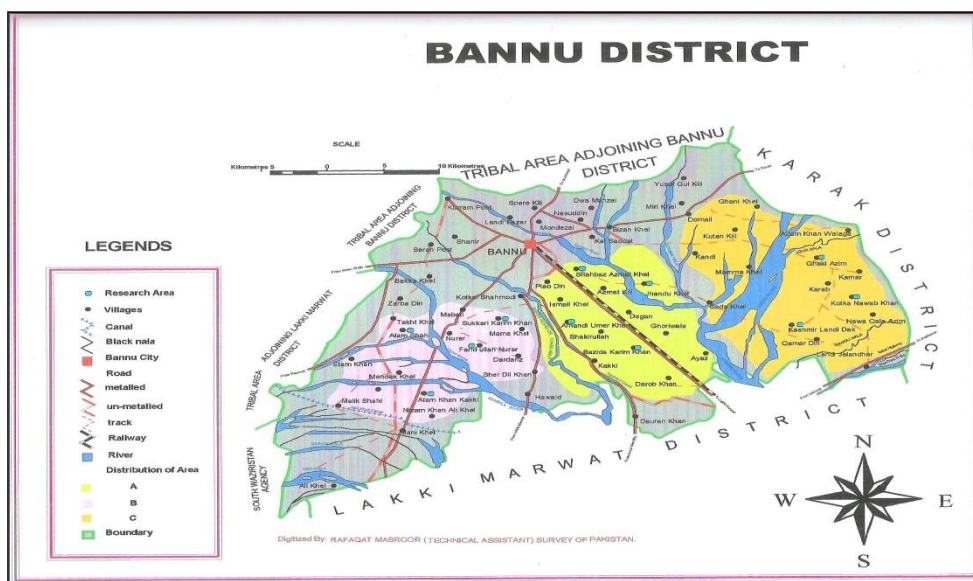


Figure 1 Map of Bannu district(using software “ArcGIS)

Selection of the Patients

Patients (Male, Female and infants) suspected for plasmodium infection were included in this study. Blood samples were randomly collected from Hospitals, Gynecology centers, Schools and Islamic Madaris.

Sample Collection

Total 440 blood samples were collected randomly from different localities both rural and urban areas of district Bannu with the assistance of Malaria Control Laboratory of the Bannu Women and Children Hospital (BWCH). During the 10 months study period the clinical history of each malaria patient was recorded on a separate data sheet.

Laboratory Diagnosis

In this study two diagnostic tests were performed; Giemsa’s stain microscopy and Rapid Diagnostic Test (RDT) according to WHO Guide lines.

Microscopic method

Slide Preparation for Microscope

Blood was drawn by pricking a finger usually from the forth finger of the left hand with the help of sterilized lancet under aseptic conditions. One lancet was used only once a time. After

accurate cleaning with spirit-moisten cotton pricking was carried out and the first blood was removed with cotton (Asif, 2008).

Thick Smear

Now a drop of blood was taken 1 cm from the right side of the slide. A separate (a smoothed edge glass) was hold at an angle of 45 degree in contact with the drop of blood, then lowered it to angle of 30 degree and pushed it gently to leave till the blood was exhausted. As the blood was exhausted the film began to form tails, which were ended near about the centre of slide (Asif, 2008).

Thin Smear

When thick smear was made then another drop of blood was taken 2.5 cm from the right side of the slide. The smooth edge spread was holded at angle of 45 degree and lowered to an angle of 30 degree to the blood. It was dragged gently to the other side and thus thin smear was made. The slides were then left to dry, followed by fixation of thin smear with methyl alcohol and labeling. The collected slides were kept in Giemsa's Stain for 20-30 minutes. These were then cleaned with tap water, dried and screened under microscope for the detection of malarial parasites (Asif, 2008).

Microscopy/ Parasite Count

After preparation of slides one drop of immersion oil was kept at the center of the smear and it was observed by binocular microscope (CX 31 Japan) under the magnification power of 100x objective. The densities of malarial parasite were also noticed per search field under microscope observation. A slide was considered negative if no parasite was seen.

Rapid Diagnostic Test (RDT)

In this test first of all cleaned the area to be lanced with an alcohol swab, and then squeezed the end of the fingertip and pierce with a sterile lancet provided, and wiped away the first drop of blood with sterile gauze or cotton. Taken a sample pipette provided, while gently squeezing the bulb, immersed the open end in the blood drop and then gently released the pressure to draw blood into the sample pipette up to the 5 µl guide line by following Mwesigwaet *al.*, 2019. The device (strip) was placed on a level surface after opening from the foil pouch, and then

transferred 5 μ l of whole blood into the sample well, and added two drops (60 μ l) of assay diluents into the diluents well. Read the test result within 20 mins

RESULTS

The epidemiological study of malaria was performed in district Bannu from September, 2013 to June, 2014. The first attempt of this research was conducted in Women and Children Hospital district Bannu, and pointed out the gender wise, age wise, and locality wise data. Total four hundred and forty (n =440) blood samples of males (n=235) and females (n=205) population were studied in various parts of district Bannu. All the samples for detection of *P.vivax*, *P. falciparum* and mixed infection were examined both by microscopy and Rapid Diagnostic test.

Microscopy Results

Distribution of malaria by species of *Plasmodium* (Microscopy) in Bannu district

By simple microscopy, 131 (29.77 %) cases were found malaria positive and 309 (70.22 %) were negative. Out of the total 131 confirmed positive cases 116 (88.54 %) samples were identified as *P.vivax* and 10 (7.63 %) as *P.falciparum*, while only 5 (3.81 %) case was reported as a mixed infection as shown in the Table 1. It was also found that malaria was most prevalent in the studied area in male's population 80 (34.04 %) than females 51 (24.87 %).

Table 1 Distribution of malaria by species of *Plasmodium* (Microscopy) in Bannu district

S. No.	Plasmodium species	Found positive	Percentage
1	<i>P. vivax</i>	116	88.54
2	<i>P. falciparum</i>	10	7.63
3	Mixed infections	05	3.81
Total		131	100

Rate of infection of malaria (Microscopy) in male and female population of district Bannu

By microscopic method total 440 samples were examined in which 131 were positive, of the total 131 positive cases 80 (34.04%) were males and 51 (24.87%) were females. It was found in the current study that malaria can affect all the age groups of both sexes. In the present work males were found to be more infected than females (Figure 2).

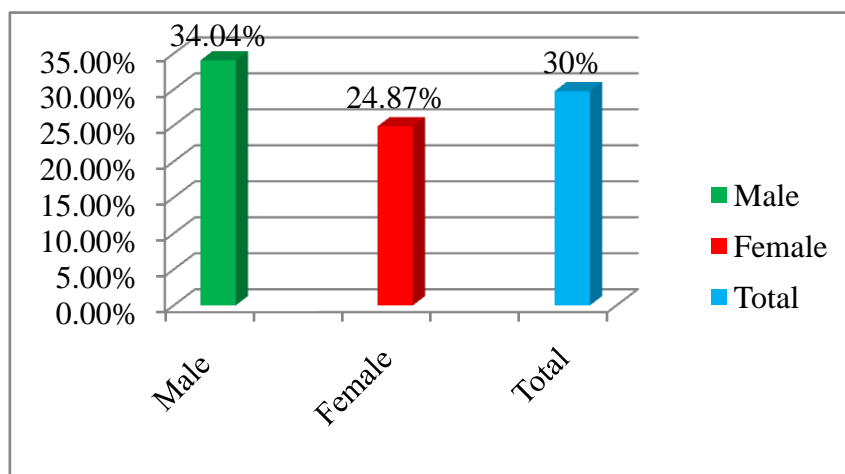


Fig 2 Rate of infection of malaria (Microscopy) in Male and female population of district Bannu

Age Wise Prevalence of Malaria (by Microscopy) in both male and female population of district Bannu

Over all prevalence of malarial parasites were found in the various age groups of male and female population by microscopy. The age groups were categorized into six groups with 10 years interval of time. The age wise prevalence was found highest (33.33%) in 1-10 years age group, while the lowest prevalence (16.66%) was found in the age group greater than 50 years. Out of 440 blood slides, 235 blood films were collected from males having total 80 (34.04%) *Plasmodium* positive, while the remaining 205 blood samples were collected from females having total 51 (24.87%) *Plasmodium* positive (Table 2).

Table 2 Age Wise Prevalence of Malaria (by Microscopy) in both male and female population of district Bannu

Age	No. of	Male	Female	Total	Total

Group (Years)	Samples					Positive%	negative%
		+ve	-ve	+ve	-ve		
1-10	204	46	76	22	60	68 (33.33%)	136(66.66%)
11-20	96	17	37	12	30	29(30.2%)	67(69.79%)
21-30	72	7	20	10	35	17(23.61%)	45(62.5%)
31-40	41	8	14	4	15	12(29.26%)	29(70.73%)
41-50	15	1	3	2	9	3(20%)	12(80%)
>50	12	1	5	1	5	2(16.66%)	10(83.33%)
Total	440	80	155	51	154	131(29.77%)	309(70.40%)

Prevalence of malaria (microscopy) in general population of district Bannu by sector wise

A general survey was made, during which 440 blood samples were collected throughout the Bannu district. After their careful examination, 131 of the total slides were found malaria positive by microscopy. During the current work, 205 samples out of 440 were collected from sector A, among which 68(33.17%) were found positive. 137 of the 440 blood samples were collected from sector "B", among which 42(30.65%) were found positive. 98 of the 440 blood sample were collected from sector "C", among which 21(21.42%) were found positive (Table 3). The sector wise prevalence showed that it was highest 68(33.17%) in sector "A" and the lowest prevalence 21(21.42%) was found in sector "C". In sector "B", the prevalence was 42(30.65%) moderate.

Table 3 Prevalence of malaria (Microscopy) in general population of district Bannu by sector wise

Sector	S.No.	Name of locality	Total no. of sample Collected	Positive sample	%tage% of whole Sector
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A	1	JhanduKhel	44	19	43.18%	33.17%	
	2	ShabazAzmatKhel	51	20	39.21%		
	3	BazidaKarim Khan	37	16	43.24%		
	4	AmandiUmer Khan	73	13	17.80%		
	Total		205	68	143.43%		
B	5	Ali Khan Kakki	33	15	45.45%	30.65%	
	6	SukkariKarim Khan	41	14	34.14%		
	7	Alam Shah	35	6	17.14%		
	8	FaridullahNurar	28	7	25%		
	Total		137	42	121%		
C	9	KotkaNawab Khan	43	7	16.27%	21.42%	
	10	Azim Kala	31	9	29.03%		
	11	Kashmir Landidak	24	5	20.83%		
	Total		98	21	63.81%		
	Grand Total		440	131	29.77%		

RDT Results

Distribution of malaria by species of *Plasmodium*(by RDT) in Bannu District

By RDT, 97 (22.04 %) cases were found malaria positive and 343 (77.95 %) were negative. Out of the total 97 confirmed positive cases 61 (62.88 %) samples were identified as *P.vivax* and 29 (29.89 %) as *P.falciparum*, while only 07 (7.21 %) case was reported as a mixed infection as shown in the Table 4. During this research, among the 97 positive malaria samples, *P.vivax* was found to be more prevalent and wide spread one 61 (62.88%) as compared to *P. falciparum* 29

(29.89%), while the rest 07 (3.81%) showed mixed infection of both *Plasmodium* species. None of the subjects in the current study had *P. malariae* or *P. ovale* infections.

Table 4 Distribution of malaria by species of Plasmodium (by RDT) in Bannu district

S. No.	Plasmodium species	Found positive	Percentage
1	<i>P. vivax</i>	61	62.88
2	<i>P. falciparum</i>	29	29.89
3	Mixed infections	07	7.21
Total		97	100

Rate of infection of malaria (by RDT) in male and female population of district Bannu

By RDTs total 440 samples were examined in which 97 were positive, of the total 97 positive cases 59 (23.98%) were males and 38 (19.58%) were females. It was found in the current study that malaria can affect all the age groups of both sexes. In the present work males were found to be more infected than females (Figure 3).

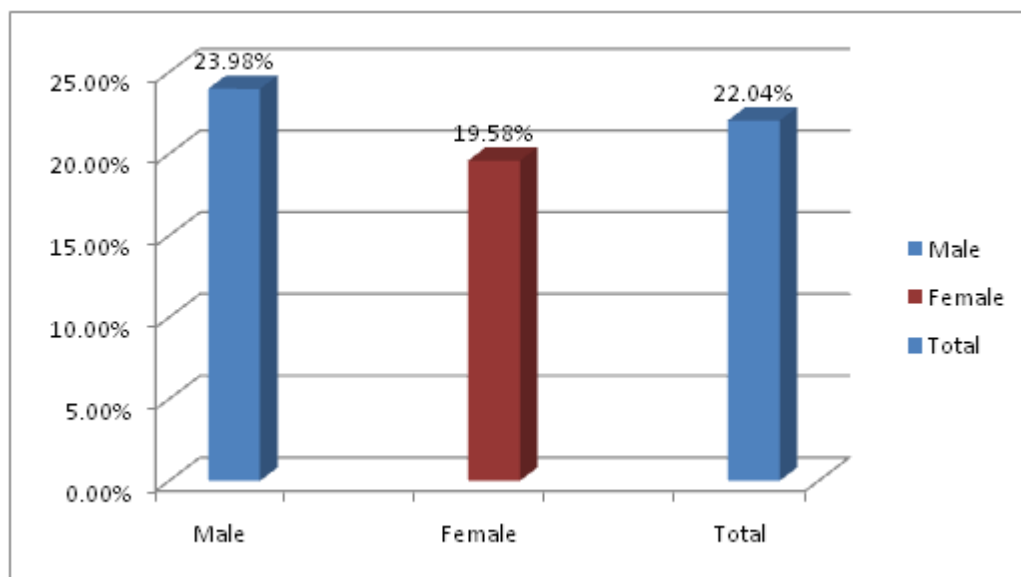


Figure 3 Rate of infection of malaria (by RDT) in male and female population of district Bannu

Age Wise Prevalence of Malaria (by RDT) in both male and female population of district Bannu

Over all prevalence of malarial parasites were found in the various age groups of male and female population by RDTs. The age groups were categorized into six groups with 10 years interval of time. The age wise prevalence was found highest (24.5%) in 1-10 years age group, while the lowest prevalence (8.33%) was found in the age group greater than 50 years. Out of 440 blood slides, 246 blood films were collected from males having total 59 (60.82%) *Plasmodium* positive, while the remaining 194 blood samples were collected from females having total 38 (19.58%) *Plasmodium* Positive (Table 5).

Table 5 Age Wise Prevalence of Malaria (by RDT) in both male and female population of district Bannu

Age Group (Years)	No. of Samples	Male		Female		Total Positive%	Total negative%
		+ve	-ve	+ve	-ve		
1-10	204	34	88	16	66	50 (24.5%)	154(75.49%)
11-20	96	12	42	9	33	21(21.87%)	75(78.125%)
21-30	72	5	22	8	37	13(18.05%)	59(81.94%)
31-40	41	6	16	3	16	09(21.95%)	32(78.04%)
41-50	15	1	3	2	9	03(20%)	12(80%)
>50	12	1	5	0	6	01(8.33%)	11(91.66%)
Total	440	59	176	38	167	97(22.04%)	343(77.95%)

Prevalence of malaria (by RDT) in general population of district Bannu by sector wise

By general survey, Total 440 blood samples were collected throughout the Bannu district. After their careful examination, 97 of the total slides were found malaria positive by RDT. During the current work, 205 samples out of 440 were collected from sector A, among which 55(26.82%) were found positive. 137 of the 440 blood samples were collected from sector "B", among which

28(20.43%) were found positive. 98 of the 440 blood sample were collected from sector "C", among which 14(14.28%) were found positive (Table 6). The sector wise prevalence showed that it was highest 55(26.82%) in sector "A" and the lowest prevalence 14(14.28%) was found in sector "C". In sector "B", the prevalence was 28(20.43%) moderate.

Table 6 Prevalence of malaria (by RDT) in general population of district Bannuby sector wise

Sector	S. No.	Name of locality	Total no. of sample collected	+ve samples	%tage	%tage of whole Sector
A	1	JhanduKhel	44	16	36.36%	26.82%
	2	ShabazAzmatKhel	51	19	37.25%	
	3	BazidaKarim Khan	37	9	24.32%	
	4	AmandiUmer Khan	73	11	15.06%	
	Total			205	55	112.99%
B	5	Ali Khan Kakki	33	11	33.33%	20.43%
	6	SukkariKarim Khan	41	8	19.51%	
	7	Alam Shah	35	5	14.28%	
	8	FaridullahNurar	28	4	14.28%	
	Total			137	28	81.4%
C	9	KotkaNawab Khan	43	3	6.97%	14.28%
	10	Azim Kala	31	7	22.58%	
	11	Kashmir Landidak	24	4	16.66%	

	Total	98	14	46.21%	
	Grand Total	440	97	22.04%	

Comparison of microscopic method with RDT method

In the current study total 440 samples were examined both by microscopy and RDT method. Out of 440 samples 131 were found positive by microscopy and 97 were found by RDTs. In microscopy the prevalence of *P.vivax*, *P. falciparum*, and mixed infection were 116/131 (88.59%), 10/131 (7.63%), and 05/131 (3.8%) respectively. While in RDT method the prevalence of *P.vivax*, *P. falciparum*, and mixed infection were 61/97 (62.88%), 29/97 (29.89%) and 07/97 (7.21%) respectively. By comparison microscopy show high prevalence rate of infection than RDT method while in mixed infection RDT show high prevalence rate of infections as shown in Table 7. In gender wise comparison microscopy show high prevalence rate of infection both in male 80/131 (34.04%) and female 51/131 (24.87%) population of district Bannu while in RDT method the prevalence rate was 59/97 (23.98%) in males and 38/97 (19.58%) in females as shown in Figur 4).

Table 7 Specious wise comparison of microscopic method with RDT method in general population of district Bannu

S. No.	Specious wise comparison		
	Plasmodium species	Found +ve (Microscopy)	Found +ve (by RDT)
1	<i>P. vivax</i>	116 (88.54%)	61 (62.88%)
2	<i>P. falciparum</i>	10 (7.63%)	29 (29.89%)
3	Mixed infections	05 (3.81%)	07 (7.21%)
Total		131/440	97/440

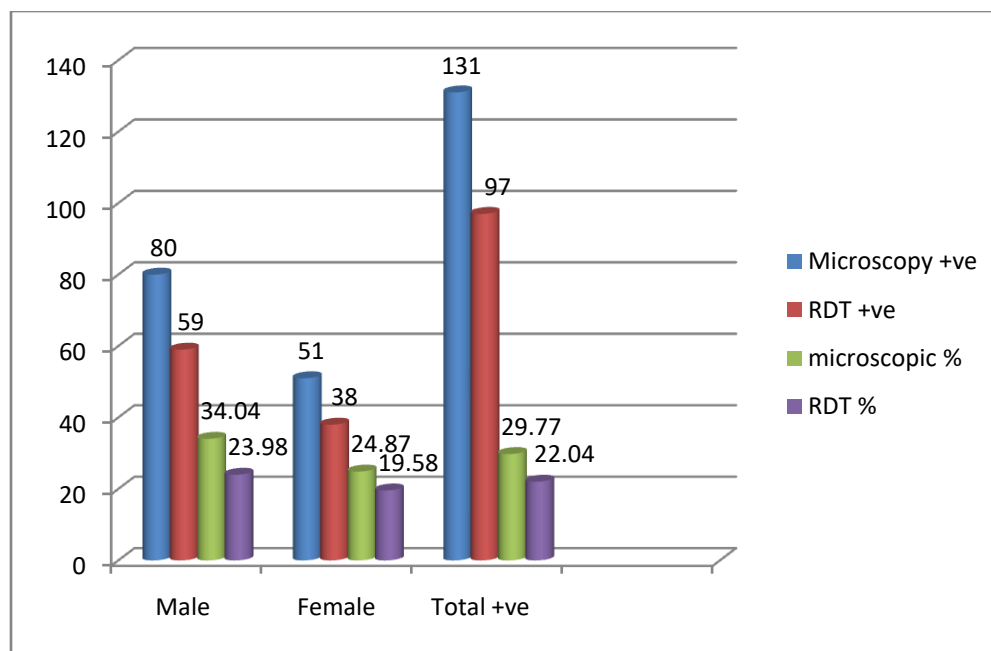


Figure 4 Gender wise Comparison of microscopic method with RDT method in general population of district Bannu

DISCUSSION

Malaria is the world's most important parasitic infection which possesses major health challenges (Mendis *et al.*, 2001). Its endemic reports today suggest wide range of morbidity and mortality among rural population as well as in those living in urban slums, where poor personal hygiene, breakdown in sanitation and socio-economic standards, tremendously contribute for the spread of the disease (Khattak *et al.*, 2013). Malaria affects people in more than 108 countries around the world mostly among children in Africa (Hay *et al.*, 2005). In Pakistan malaria infection is moderately endemic. In prevalence there is variation from area to area and province to province (Khatoun *et al.*, 2010). The only prevalent species of malaria parasite are *P. vivax* and *P. falciparum* in the country but *P. vivax* was the leading species accounting for more than 70% of the malaria trouble in the country. The highest trouble of vivax malaria was reported from Khyber Pakhtunkhwa (KP) and the FATA (Federally Administered Tribal Areas), illustrating the need for greater programmatic and health system strengthening in these regions (Murtaza *et al.*, 2009).

Malaria is a major public health problem in Pakistan and, along with other “Category 3” countries (Somalia, Sudan, Yemen, Afghanistan, Djibouti and Pakistan) of the Eastern

Mediterranean Region (EMR) account for more than 95% of the regional burden (Huda and Zamani, 2008). In the current study general epidemiology and identification of malarial parasites was carried out by microscopy and RDT to know the high prevalence status of malaria infection. The rate of positive slide in this study was 131/440 (29.77%) in case of microscopy and RDTs positive cases were 97/440 (22.04%). In this study, the prevalence of *P. vivax* was observed to be higher (88.54%) as compared with that of *P. falciparum* (7.63%). Similarly, Yaret *et al.* (1998) while studying incidence of malarial parasite species in Multan district, observed high prevalence of *P. vivax* (60.50%) and a low incidence of *P. falciparum* (37.20%). Similarly, Jan and Kiani (2001) while studying malarial parasites in Kashmiri refugees settled in Muzaffarabad reported high incidence (6.33%) of *P. vivax* than of *P. falciparum* (0.67%). Mohammad and Hussain (2003) observed high incidence of *P. vivax* (5.78%) and 1.08% *P. falciparum*. Malaria in Karachi and other areas in Sindh were studied by Mahmood (2005) who observed *P. vivax* to be two times higher than *P. falciparum*. Idris *et al.* (2007) while studying pattern of malarial infection at Ayub Teaching Hospital, Abbottabad found that out of 1994 patients screened, 145 (7.2%) were found infected. *P. vivax* was seen in the majority (72.4%) than *P. falciparum* (24.1%). Similar studies conducted by Sheikh *et al.*, 2005 in Quetta the prevalence of *P. vivax* was higher 66.8 % than *P. falciparum* 30.7% and Khan *et al.*, 2006 in D.I.Khan found out that the prevalence of *P. vivax* was lower 40.8 % than the *P. falciparum* 57.1 %. The difference in the studies might be due to variation in sample size, age and geographical conditions. In the present survey *Plasmodium vivax* was more prevalent than *Plasmodium falciparum*, which is possibly due to no second exothermic cycle and true relapses do not occur in *P. falciparum*, whereas in *P. vivax* relapses are present (Muhammad and Hussain. 2003).

In the current study males were more vulnerable to malaria infection 80/131 (34.04% by microscopy) and 59/97 (23.98% by RDT) than females 51/131 (24.87 % by microscopy) and 38/97 (19.58% by RDT) confirmed by both diagnostic methods. In other similar study by Khan *et al.*, 2006 in D.I.Khan malaria infection was also more in males 75/98 (76.5 %) than females 23/98 (23.4 %). This difference in variation of infection might be due to the areas custom and tradition. In our study area males were traditionally more exposed to environment as compared to females, because males go to the field for working in different conditions while females are mostly restricted to their homes and well covered as compared to males.

It was found in the current study that malaria can affect all the age groups of both sexes. In the current study the highest prevalence 68(33.33%) was found in 1-10 years age group by microscopy and the lowest prevalence 2(16.66%) was found in age group > 50 years, through RDT the highest prevalence 50(24.55) was found in 1-10 years age group and the lowest prevalence 2(16.66%) was found in age group 50< years. Similarly Awan and Jan (2008), found the highest prevalence in 5 – 10 years age groups (13.33%) and lowest prevalence (4.62%) was found in age group 46 – 50 years. . Similarly malaria infection varied in various age groups 50.4 %, 28.6 %, 25.0 %, 7.1 % and 26.3 % respectively in 11 to 20, 21 to 30, 31 to 40, 41 to 50 years by microscopy (Tomaet *al.*, 2001). In the present study slight variation of infection rates were observed in the varying age groups , 33.33 %, 30.2 %, 23.61 %, 29.26 %,20%,16.66% by microscopy which may be due sleeping location, sanitary condition, sex difference, area also play a vital role, weather has also its remarkable importance. The prevalence of malaria was highest both by microscopy (33.17%) and RDT (26.82%) in sector A while the lowest prevalence of malaria infection was found in a sector C. It might be due to the poor sanitary condition, the presence of marshy and stagnant water in this sector.

The prevalence of malaria observed during the present survey (29.77%) is higher than the prevalence recorded (17.35%) in general population of Bannu district (Khan *et al.*, 2013). This high incidence may be due to the inadequate health-care facilities, unawareness about good living hygienic conditions and poor sanitary conditions.

CONCLUSION

In this study a high prevalence status of malaria infection was found in the general population of district Bannu. *P.vivax* was highly epidemics as compared to *P.falciparum*, while no other infection of *P.malariae* and *P.ovale* were found in the study area. For general Laboratory detection, microscopic method was a suitable tool for malarial parasites as compare to RDT method.

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REFERENCES

1. Asif, S. A. (2008). Departmental audit of malaria control programme 2001-2005 north west frontier province (NWFP). *J Ayub Med Coll Abbottabad*, 20(1), 98-102.
2. Atta, H., & Zamani, G. (2008). The progress of Roll Back Malaria in the Eastern Mediterranean Region over the past decade. *EMHJ-Eastern Mediterranean Health Journal*, 14 (Supp.), S82-S89, 2008.
3. Carlton, J. M., Adams, J. H., Silva, J. C., Bidwell, S. L., Lorenzi, H., Caler, E., ... & Fraser-Liggett, C. M. (2008). Comparative genomics of the neglected human malaria parasite *Plasmodium vivax*. *Nature*, 455(7214), 757-763.
4. Carter, R., & Mendis, K. N. (2002). Evolutionary and historical aspects of the burden of malaria. *Clinical microbiology reviews*, 15(4), 564-594.
5. Drakeley, C., & Reyburn, H. (2009). Out with the old, in with the new: the utility of rapid diagnostic tests for malaria diagnosis in Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 103(4), 333-337.
6. Greenwood, B. M., Fidock, D. A., Kyle, D. E., Kappe, S. H., Alonso, P. L., Collins, F. H., & Duffy, P. E. (2008). Malaria: progress, perils, and prospects for eradication. *The Journal of clinical investigation*, 118(4), 1266-1276.
7. Hamid, A., & Tanvir Ahmad, K. (2001). Haematozoan parasites in Kashmiri refugees.
8. Hay, S. I., Guerra, C. A., Tatem, A. J., Atkinson, P. M., & Snow, R. W. (2005). Urbanization, malaria transmission and disease burden in Africa. *Nature Reviews Microbiology*, 3(1), 81-90.
9. Khan, A. K., Shah, A. H., Suleman, M., & Khan, M. A. (2012). Assessment of Malaria Prevalence Among School Children In Rural Areas of Bannu District Khyber Pakhtunkhwa, Pakistan. *Pakistan Journal of Zoology*, 44(2).
10. Khan, H. U., & Khattak, A. M. (2006). A study of prevalence of malaria in adult population of DI Khan, Pakistan. *Biomedica*, 22(14), 99-104.
11. Khatoon, L., Baliraine, F. N., Bonizzoni, M., Malik, S. A., & Yan, G. (2010). Genetic structure of *Plasmodium vivax* and *Plasmodium falciparum* in the Bannu district of Pakistan. *Malaria Journal*, 9(1), 1-10.
12. Khattak, A. A., Venkatesan, M., Nadeem, M. F., Satti, H. S., Yaqoob, A., Strauss, K., ... & Plowe, C. V. (2013). Prevalence and distribution of human *Plasmodium* infection in Pakistan. *Malaria journal*, 12(1), 1-8.
13. Mendis, K., Sina, B. J., Marchesini, P., & Carter, R. (2001). The neglected burden of *Plasmodium vivax* malaria. *The American journal of tropical medicine and hygiene*, 64(1_suppl), 97-106.
14. Ministry of Health: *Epidemiology of malaria in Pakistan*. (2010).
15. Muhammad, N., & Hussain, A. (2003). Prevalence of malaria in general population of district Buner. *Journal of Postgraduate Medical Institute (Peshawar-Pakistan)*, 17(1).

16. Murtaza, G., Memon, I. A., Memon, A. R., Lal, M. N., & Kallar, N. A. (2009). Malaria morbidity in Sindh and the plasmodium species distribution. *Pak J Med Sci*, 25(4), 646-649.
17. Mwesigwa, J., Slater, H., Bradley, J., Saidy, B., Ceesay, F., Whittaker, C., ... & D'Alessandro, U. (2019). Field performance of the malaria highly sensitive rapid diagnostic test in a setting of varying malaria transmission. *Malaria journal*, 18(1), 1-13.
18. Palmer, C. J., Bonilla, J. A., Bruckner, D. A., Barnett, E. D., Miller, N. S., Haseeb, M. A., ... & Stauffer, W. M. (2003). Multicenter study to evaluate the OptiMAL test for rapid diagnosis of malaria in US hospitals. *Journal of clinical microbiology*, 41(11), 5178-5182.
19. Rubio, J. M., Benito, A., Berzosa, P. J., Roche, J., Puente, S., Subirats, M., ... & Alvar, J. (2005). Usefulness of seminested multiplex PCR in surveillance of imported malaria in Spain. *Journal of Clinical Microbiology*, 37(10), 3260-3264.
20. Sachs, J., & Malaney, P. (2002). The economic and social burden of malaria. *Nature*, 415(6872), 680-685.
21. Snow, R. W., Guerra, C. A., Noor, A. M., Myint, H. Y., & Hay, S. I. (2005). The global distribution of clinical episodes of *Plasmodium falciparum* malaria. *Nature*, 434(7030), 214-217.
22. Stauffer, W. M., Cartwright, C. P., Olson, D. A., Juni, B. A., Taylor, C. M., Bowers, S. H., ... & Boulware, D. R. (2009). Diagnostic performance of rapid diagnostic tests versus blood smears for malaria in US clinical practice. *Clinical Infectious Diseases*, 49(6), 908-913.
23. WHO, World Malaria Report 2010; December 2010.3 CDC, Malaria.
24. World Malaria Report 2009. Geneva, World health Organization, 2009.
25. Yar, H. M., Masood, K., Maqbool, A., & Malik, G. Q. (1998). Prevalence of malarial parasite species in Multan district. *The Professional*, 5, 183-7.
26. Yasinzai, M. I., & Kakarsulemankhel, J. K. (2009). Prevalence of human malaria infection in District Ziarat and Sanjavi, Pakistan. *Pakistan Journal of Zoology*, 41(6).
27. Zakeri, S., Kakar, Q., Ghasemi, F., Raeisi, A., Butt, W., Safi, N., ... & Djadid, N. D. (2010). Detection of mixed *Plasmodium falciparum* & *P. vivax* infections by nested-PCR in Pakistan, Iran & Afghanistan. *Indian J Med Res*, 132, 31-35.
28. Zakeri, S., Safi, N., Afshar, M., Butt, W., Ghasemi, F., Mehrizi, A. A., ... & Djadid, N. D. (2010). Genetic structure of *Plasmodium vivax* isolates from two malaria endemic areas in Afghanistan. *Acta Tropica*, 113(1), 12-19.
29. Zia-ur-Rahman, A., & Jan, A. H. (2008). Rice fields in relation to malaria in district Bannu NWFP, Pakistan. In *Proceedings of Pakistan Congress of Zoology (Pakistan)*.