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Complete blood count (CBC) are closely correlated with *Plasmodium vivax* density in district Bannu, Khyber Pakhtunkhwa, Pakistan

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Abstract
The current study was conducted in Women and Children Teaching Hospital (WCTH) district Bannu to correlate the *Plasmodium vivax* density on complete blood count (CBC). The study contained 137 blood samples of patients, who visited to the hospital with the complaints of fever, headache, abdominal pain and shivering. Among these 49 (35.766%) were found positive for *P. vivax* and 88 (64.233%) were found negative. Among the positive cases the male and female was recorded 65.306% and 34.693% respectively. The patients, who have less than ten years of age, were more infected by *P. vivax*. The maximum density in patients was recorded four per field in microscope but the density two were more abundant. The parasite density decreased with the increase in WBCs count. Similarly the platelets count increases when parasite density decreases. The maximum *P. vivax* density reduce the WBCs and platelets count which may weaken the immune system and the person exposed to other pathogens like salmonella, hepatitis viruses and other diarrhea causing agents etc. Furthermore the study helps us for the improvement of malaria control and other strategic plan among the population of district Bannu.

Keywords: Complete blood count (CBC), correlation, *Plasmodium vivax*

1. Introduction
District Bannu is situated in between the 31.28^0 North latitude and 73.25^0 East longitudes. It is located in the southern region with its borders contain districts Karak, Lakki Marwat and the North South Waziristan Agencies. According to 2017 Census the estimated population of district Bannu is 1,167,892 with annual growth rate of 2.81% respectively. The total area of district Bannu is 1,227 square kilometers, but the cultivated area is 74196 Hectors. The climate is warm in summer (48 ^{0}C) and cooled in winter (6 ^{0}C) season. 45% of area is irrigated through canal systems, while the remaining area is depend upon the rain fall. Malaria is most common infection of about more than 200 million peoples involved worldwide [1]. Four species of the plasmodium are documented in spreading of malaria namely, *Plasmodium falciparum, Plasmodium malariae, Plasmodium vivax* and *Plasmodium ovale*. But the *P. falciparum* is most common and most threatened species [2]. It is the most general source of disease, about 80% of all malarial infections and about 90% cause deaths from malaria [3]. Malaria pathogenesis is very difficult and entails immunologic and non-immunologic mechanisms [4].
In Pakistan *P. vivax* and *P. falciparum* are most common [5-6]. Approximately 75% and 25% peoples are infected from *P. vivax* and P. *falciparum* infection respectively [7]. World Health Organization (WHO) documented that 150 million, 97% of Pakistani suffer from malaria and determined the nationwide of 1.6 million cases per year [8]. The epidemiology of malaria in Pakistan is generally seasonal in most areas like Khyber Pakhtunkhwa, Baluchistan and the Sind provinces. Malaria is transmitted abundantly in September, November and during monsoon but less in spring season especially March and April. Most cases during spring are dormant in the liver and appeared after the monsoon season especially *P. vivax* infection [7].
Platelets play very vital role in the plasmodium infections pathogenesis which encourages the confiscation of infected erythrocytes within the vasculature of brain. It also plays critical roles in native protection against pathogens infections.

Platelets also bind with plasmodium infected erythrocytes and kill it; these prove that platelets also perform the function of protection in human body. These protections of platelets occur in the early stages of the red blood cells infection which is broadly different from the cerebral malaria [9]. Thrombocytopenia is greatly produced in *P. falciparum* infection, while it showed low amount in *P. vivax* infection [10].

White blood cells (WBCs) counts do not reduce in different species of the plasmodium infection. Different types of methods are documented for the determination of blood parasites densities by using microscope, but the most general method is to count different range of asexual parasites in respect to the given count of WBCs. These types of determination are applied in clinical, mode of transmission studies and assessment of the effects of intervention on peoples [11]. Total leucocytes count (TLC) and differential leucocytes count (DLC) are the fundamental and important indicators in any type of diseases resulting from infection. But in malaria, the WBC ranges are generally reduced from normal during treatment [12]. The current study was aimed to correlate the *Plasmodium vivax* density with complete blood count (CBC) in district Bannu.

Materials and Methods

The present study was conducted in Women & Children Teaching Hospital (WCTH) Bannu to correlate the plasmodium vivax density on complete blood count (CBC). For this study those individuals were selected who visited the hospitals with the complaints of fever, headache, abdominal pain and shivering.

Slides preparation

From each patient 2 mL blood was taken in a sterile syringe and analyzed in hospital laboratory for WBCs count and two slides (thick smear and thin smear) were prepared. Thick smear was used for malaria parasites, while thin smear was used for WBCs count, and were stained with Giemsa's stain.

Microscopy/parasite count

After preparation of the slide one drop of emersion oil was kept at the center of the thick smear and was observed by the Japan Olympus microscope at the lens power of 100X. The parasite density was 2 to 4 per field but majority of slides were *P. vivax* with ring stage.

Platelets count

0.5mL of blood was taken with pipette and added the platelets dilution solution (Sodium citrate: 3.8 g, Formal dehyde: 0.2 ml, Brilant crazel blue: 0.1g, Water 100 ml) OR (Ammonium oxalate 1%) at the upper end of the pipette, and kept five minutes for lyses of RBCs. And the solution were added to the count chamber, and focused by Olympus microscope at the lens of power 40X. And the platelets were counted in five RBCs chambers, and total counted were multiplied by 10,000 so this was the total platelets count of any patient.

WBCs count

Total leucocytes count (TLC)

Venous blood (2 mL) was taken in disposable syringe from the patient and put in test tube contained few drops of ethylene diamine tetra acetate (EDTA). 0.5 mL of blood was taken by the WBCs pipette and then added WBCs solution (Acetic acid: 3mL, Gention Vialit: 2drops, D\water: 1000mL) at the upper end of the pipette and kept for five minutes. Because this solution was added for the lyses of RBCs added to the count chamber and then focused by the microscope at the lens of power 40x. WBCs were counted in four rectangular chambers, and then multiplied by 50 it was the TLC of any patient.

Differential leucocytes count (DLC)

One drop of blood was taken and formed a thin smear with the range of 25 to 30 mille meter length and 20 mille meters in width, then allowed to stain with gemsa stain. Kept the emersion oil at the middle of the film and focused on Olympus microscope at lens of power 100X. During observation the different leucocytes was counted by teli hand counter machine. This thin smear was consisting of neutrophill, lymphocyte, monocyte, eosinophill, and basophile. Basophile were totally absent in a smear because of low amount. But neutrophill and lymphocytes were very much in a smear, but monocyte and eosinophill were rare in a smear.

Results

A total of 137 blood samples of the suspected patients were analyzed. Among these 49 were positive for *P. vivax* and 88 were negative. Among the positive cases 32 male and 17 female were detected with variable number of ring stage of *P. vivax*. Similarly among the negative cases the male and female were 38 and 50 respectively (Figure 1).

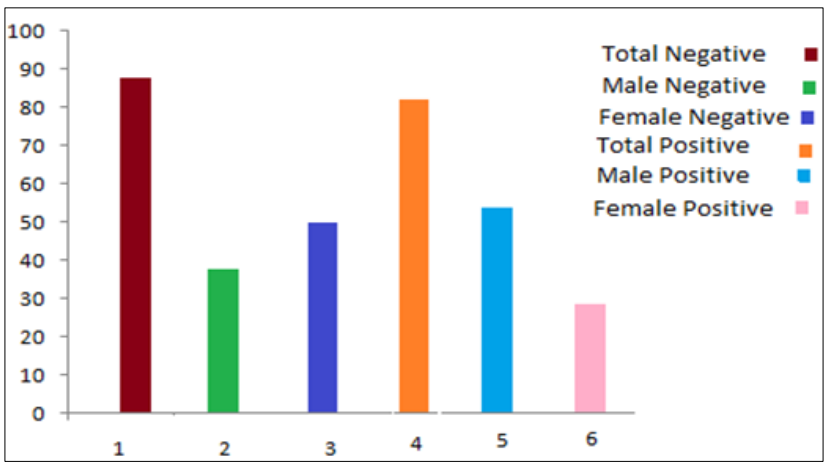

Fig 1: Sex wise prevalence of *P. vivax*

The percentile ranges of positive cases were 35.766%, male and female were 65.306% and 34.693% respectively. Similarly the negative cases were 64.233%, male and female were 43.181% and 56.818% respectively (Figure 2).

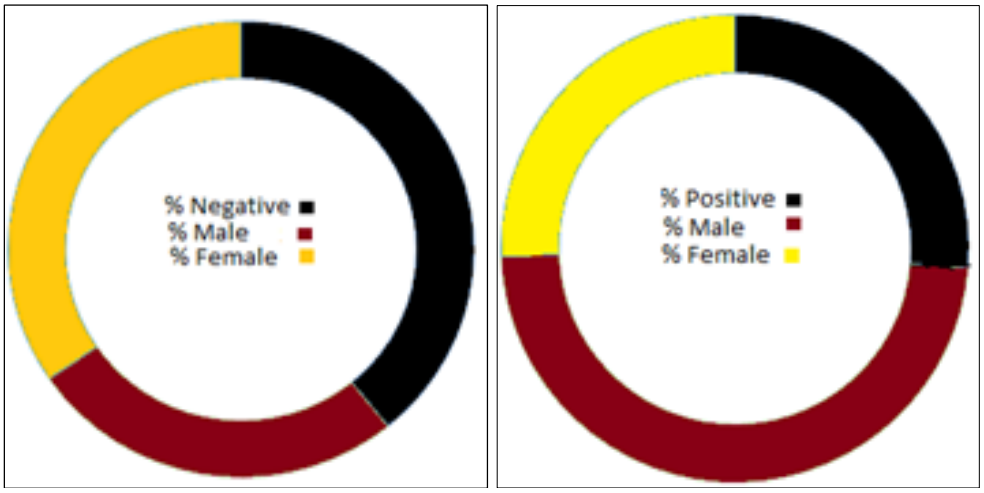

Fig 2: Percentile sex wise prevalence of *P. vivax*

The age of the individuals for this study were divided in to six groups according to their age with the lower age group less than ten year and the upper age group more than 50 years of age range. Others were divided into four groups with ten years gap. But the age groups less than 10 year is most susceptible to *P. vivax*, with male were 28 and female were 12 and other age groups were less susceptible to *P. vivax* (Figure 3).

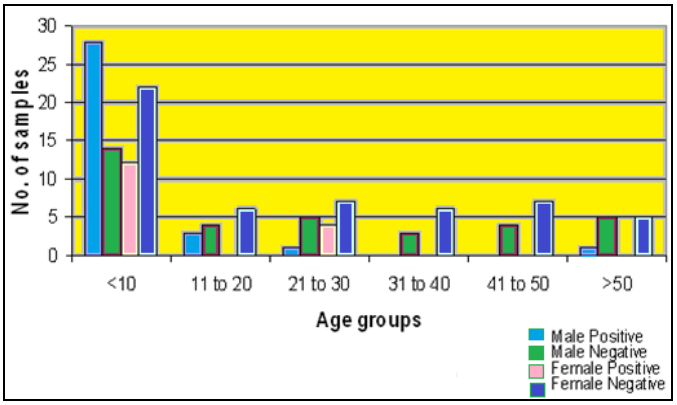

Fig 3: Age wise prevalence of *P. vivax*

The maximum *P. vivax* density was 4 and minimum density was 1. But in 11 patients the density was one and in 21 patients the density was 2. Similarly in 8 and 9 patients the density was 3 and 4 respectively. A change occurs in the WBCs count, when *P. vivax* density increases the WBCs count decreases but it decreased while the density of *P. vivax* was 2. Plasmodium density was also correlated with platelets count and it was noted that platelets count decreased when the *P. vivax* density increased (Table 1).

Table 1: WBCs and platelets count mean correlation with plasmodium densities with number of patients (n*)

n*	*Plasmodium vivax* density	White blood cells count (Mean)	Platelets count (Mean)
11	01	114818.19	254545.46 (11)
21	02	11876.19	243571.43 (21)
08	03	13350.00	248125.00 (8)
09	04	12044.45	239555.56 (9)

WBCs count was noted for every patient and the WBCs range were divided into different categories, fixed with 2000 Cu.mm gap. Started from 8000 up to the >16001, the category 10001-12000 were recorded in 19 patients which was higher. In 2 and 3 patients were recorded as the category >16001 and 14001-16000 respectively. But in most of the patients the WBCs count were found to be slightly increased than normal (Table 2).

Table 2: White blood cells counts with number of patients

n*	White blood cells count (Cu.mm)
09	8000-10000
19	10001-12000
16	12001-14000
03	14001-16000
02	>16001

Plasmodium density was also analyzed with neutrophill count but there are no significant differences found. In eosinophill there was slightly changed occur in their mean of plasmodium densities. But densities one and three have 0.82 and 0.63 mean while the remaining densities have 1.10, 1.87 mean respectively. In monocyte the 2, 3 and 4 plasmodium densities have 1.34, 1.13 and 1.78 respectively, while the remaining one plasmodium density have 0.55 mean respectively. Similarly in lymphocyte the plasmodium density was analyzed and found that when plasmodium density increased the lymphocyte count also increased (Table 3).

Table 3: Different white blood cells count correlation with plasmodium densities

n*	*Plasmodium vivax* density	N. C (Mean)	E. C (Mean)	M. C (Mean)	L. C (Mean)
11	01	54.28	0.82	0.55	41.46
21	02	54.05	1.10	1.34	44.34
08	03	48.38	0.63	1.13	49.75
09	04	51.00	1.87	1.78	48.23

(N. C: Neutrophill count, E. C: Eosinophill count, M. C: Monocyte count, L. C: Lymphocyte count)

Discussion

Malaria is a major health problem, which are strongly linked

with morbidity and mortality in the tropics regions of the Pakistan. Especially in district Bannu the *P. vivax* is more prevalent then the *P. falciparum.* A study was conducted by [13] from district Bannu and collected total of 823 blood samples, 223 (27.1%) were positive with *P. vivax* and *P. falciparum* but the *P. vivax* is more prevalent then *P. falciparum* with ranges of 186 (22.6%) and 25 (3.04%) respectively. In children of both sexes male and female the malaria infection are more as compare to the adults in district Bannu, because they are careless and slept outdoor. According to another study was conducted by [14] the male and female of age (5-10 years) infection rates were 7.18% and 6.66% respectively. Similarly the present study was same because the children of age (5-10 years) infection rates were higher as compare to the adults. The children have low immune system as compare to the adults, these malarial infections also effect on WBCs and as result of the above reports we studied the *P. vivax* density on WBCs and platelets count. The present study suggested that most of the patients have low WBCs count with increase of the plasmodium density. According to another study by [11] in which the WBCs were count in 4697 patients in Thailand of Tak Province, in between May to August 1998, and May to July 1999. At each year the WBCs counts were significantly lower in the *P. falciparum* then *P. vivax* infection and *P. vivax* have lower WBCs count than those in the uninfected patients. The population studies estimate that parasite densities on the basis of 8000 cells/μL of WBCs count. In India by [15] and reported that the *P. falciparum* and *P. vivax* infected patients had no difference between mean WBCs counts.

According to another study by [16] and 93% patients had low platelets count in *P. falciparum* infection. Similarly in Liberia, study was conducted by [17] a total of 145 patients had suffered from *P. falciparum* and *P. vivax* infections, among these, 109 (75.18%) had thrombocytopenia. The same results have also been reported in Thailand by [18, 19] the thrombocytopenia has also showed in *P. falciparum* and *P. vivax* infected patients. Another study was conducted by [20] reported that the platelets had significantly low count in *P. vivax* infection. A study was documented by [21] studied that 85.5% (171) patients with malaria had low platelets count, while 70.5% (145) patients had mild thrombocytopenia, 10.5% (21) had moderate and 4.5% (9) had severe thrombocytopenia, while 14.5% (29) (14.5%) had normal platelet count. But our study revealed that *P. vivax* density had significantly low mean platelets count which causes moderate thrombocytopenia.

Conclusion

Plasmodium density is the number of parasite per field under microscope. So it was concluded that plasmodium density have greatly affected the WBCs and platelets counts. The maximum *P. vivax* density reduce the WBCs and platelets count which may weaken the immune system and the person exposed to other pathogens like salmonella, hepatitis viruses and other diarrhea causing agents etc. Furthermore the study helps us for the improvement of malaria control and other strategic plan among the population of district Bannu.

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