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Prevalence of plasmodium infection in district Multan, Punjab, Pakistan

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Abstract
Background: *Plasmodium* infection (Malaria) always remains hazardous for its fatalities worldwide, especially for Pakistan; it is yet endemic in many areas. Out of five types of *Plasmodium, Plasmodium vivax* and *Plasmodium falciparum* is dominating in Pakistan. *P. vivax* is more frequent and widely distributed while *P. falciparum* is more dangerous and deadly in action. This research was conducted for the assessment of *Plasmodium* infection frequency in human populations of District Multan, Punjab, Pakistan.
Methods: *Plasmodium* type was identified in the blood of high graded fever patients. Immuno chromatographic Technique (ICT) as well as thick and thin blood smears procedure was performed at different laboratories of district Multan where the microscopic examination was carried out for the presence of *Plasmodium* types and its species.
Results: Statistically, the current prevalence and distribution of *Plasmodium* types was estimated in this study. A total of 192 blood samples were examined. The *Plasmodium* species recovered were *P. vivax, P. falciparum* and mixed species. The prevalence of *P. vivax, P. falciparum* and mixed species was 13.02%, 10.41% and 1.041% respectively.
Conclusion: *Plasmodium vivax* had prevalence over Plasmodium *falciparum.* The prevalence species was more in males as compared to females. The age-wise prevalence of plasmodium infection showed that it was more prevalent in patients of age group 21-30 years.

Keywords: ICT, thick and thin smear, *Plasmodium vivax, P. falciparum*, prevalence

Introduction
It is well known fact that malaria has killed more people than any other disease. Populations are at increased risk for severe malaria as WHO estimated that annually three hundred to five hundred million cases of malaria occur and more than 1 million people die of malaria. As per WHO report of 2015, around 3.2 billion individuals stay in danger of *Plasmodium* infection. 214 million new patients of *plasmodium* infection were registered and 438000 fatalities. A huge number of individuals are even not getting the remedies they have to avoid and treat *Plasmodium* infection [1]. WHO reported that nearly 82% of *P. vivax* cases in 2017 only registered in Pakistan and other four South Asian Countries [2].
Malaria is a mosquito-borne infection spread by an intracellular protozoan parasite called *Plasmodium.* The word malaria comes from the Italian words' "mala" means bad and "aria" means air in the 18th century as considered to be spread by dirty emission from the marshy soil. It was idea that the stagnant water of swamps harmed the air and because of inhaling this "bad air", human being received malaria [3].
Sir Ronald Ross proved the relation between mosquito and malaria. About 25000 mosquitoes were dissected by Sir R. Ross in his life. He established that female Anopheles is the carrier of *Plasmodium.* He enabled a female to nibble a malarial patient. He died the mosquito after a few days and discovered plasmodium duplicating in mosquito's stomach. He is awarded by Noble Prize on 29th August in 1902. This day is celebrated as malaria day [4]. Cinchona bark had been proved amazing for curing malaria. It is because that cinchona bark consists of quinine that is powerful in curing the malaria [5].
Malaria is one of the serious health dangers to a large number of peoples of the tropical and subtropical regions of the world. It has overpowering the developing world and causes more than one million deaths each year [6]. Malaria found worldwide but mostly connected to tropical and sub-tropical areas and an altitude below 1500 m. *P. vivax* is more common in

Central America and the Indian subcontinent [7]. Almost 50 % cases of *P. falciparum* are found in Africa while in other parts of the world its occurrence is only 10 % [8]. Malaria is contiguous infection in Pakistan because of adverse hot climate. Outburst happens from June to November connecting with rainstorm season. Heavy rainfall, outflow and poor sanitation and drainage, inadmissible administration of waste and refuses and minimum medicinal services particularly in villages, give a position of shelter to the spread of the mosquito vectors of malaria and dengue fever, appropriately commonness of these diseases are greater in rural area as compared to urban areas [9]. In Pakistan, 80% of the populace is at risk, putting immense burden on financial condition of the country. Nearly 60% of Pakistan's population, live in malaria endemic localities [10].

Material and Method

Study Area

Multan is the oldest, hotter and historical city of Pakistan. Multan is also known as the city of the Saints. Multan is located in the south of the province, Punjab, Pakistan. Multan is the central place of Southern Punjab. It consists of four Tehsil comprises of round about 5 million people. Multan dependably lies at real danger of Plasmodium infection and crosses its top during overwhelming rain falls through July and August. An examination of commonness of *Plasmodium* infection in the people of District Multan was conducted from March to October 2018. Age of infected persons ranges up to 70 years, including males and females.

Patient & Sample Selection

Totally, 192 Blood Samples were randomly collected from high graded fever patients in various laboratories, of District Multan. Blood tests were taken from the people with consent who had malarial fever symptoms such as high fever, severe head pain, nausea and vomiting. Observations were done to collect data regarding sex, age, season and species. Blood samples were taken from various hospitals and labeled appropriately. This study was completed at laboratories of various hospitals of District Multan, Pakistan from March 2018 to October 2018. Multan with hotter climate, open and the poor drainage system, and existence of dirty ponds and lakes are a major and a favorite source for the spread of mosquito. The whole patients admitted in various hospitals with high graded fever and having the same symptoms of malaria were included in this studied. Blood samples were collected from Hospitals of Multan:-

1. Nishtar Hospital, Multan
2. Govt. THQ Hospital Shujabad
3. Govt. Mushtaq Laang THQ Hospital, Jalalpur pirwala
4. Multi Care Complex, Nishtar Road, Multan.

Identification of Plasmodium by ICT

For identification of *Plasmodium* ICT assay method was utilized in *Plasmodium* identification. In this technique the EDTA whole blood is taken for microscopy and ICT malaria fit for *P. falciparum* identifying particular histidine rich protein II Antigen(PfHRP-II) and *P. vivax* particular lactate dehydrogenase (Pv. LDH) in patient blood. From this examination, this is concluded that ICT rapid method can be utilized as substitute to blood smear preparation for the detection of *Plasmodium* in such localities where peripheral blood film method is not accessible [11].

Whole blood sample method

In whole blood sample method, a drop of blood is taken, and 5 drops of buffer are added to it. This technique also confirms the reacted types of *Plasmodium.*

Blood Smear

In this technique, a small drop of whole blood was placed from one side on a Very clean slide. After this, hold a second slide and maintained contact with the lower slide. Then pulled the upper slide back to contact the drop, which would spread by capillary action. It maintained firm contact with the lower slide and push the top slide in one motion to produce the smear.

Preparation of thin & thick blood smears

Thin peripheral blood film can be prepared from anti-coagulated blood obtained by venipuncture or from free flowing finger prick blood. A small drop of blood is placed in the middle of a clean glass slide. Another slide (spreader) with smooth edge at an angle of 30-45^0 was taken near the drop of blood. Move the spreader backward so that it made contact with drop of blood. Then moved the spreader forward rapidly over the slide. A thin peripheral blood film was thus prepared. Dry and stained it.

Thick blood smear was used to detect the presence of Plasmodium while thin blood smears helped to find which types of Plasmodium was the source of malaria. In this technique placed a large drop of blood in the center of a clean glass slide. Spread it in a circular area of 1.5 cm with the help of a stick or end of another glass slide. Dry and stained it. Thick blood smear was not fixed in methanol while thin smear fixed in methanol.

Prevalence Rate

Prevalence was calculated with the help of following formula.

$$\text{Prevalence Rate: } \frac{\text{No. of patient's having malaria}}{\text{Total No. of patient's diagnose}} \times 100$$

Results

Total number of tests with percentages

It was found that out of total 192 high graded fever patients which were examined, 145 samples (75.52%) were found negative for plasmodium while only 47 samples (24.48%) were detected positive for *Plasmodium*. The Results has shown in figure 1.

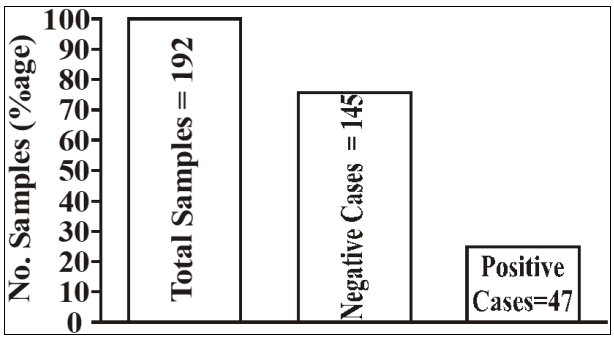

Fig 1: Plot showing of total number of samples & number of positive and negative cases and their percentages.

Gender wise result

Out of total 192 tests, 105 suspected males with high graded fever ranging between 100 to 105^{0}F, chills, vomiting, profuse

sweating, anemia, muscle pain and shivering were examined, out of which 29 (27.61%) males were found positive and 76 were found negative for *Plasmodium*. Both ICT & thick and thin film methods were performed for the confirmation of positive stains. Positive tests were further confirmed by ICT device. While out of 192 samples, 87 female's patients with high graded fever were examined, out of which 18 (20.68%) females were found positive and 69 were negative for *Plasmodium* (Fig. 2).

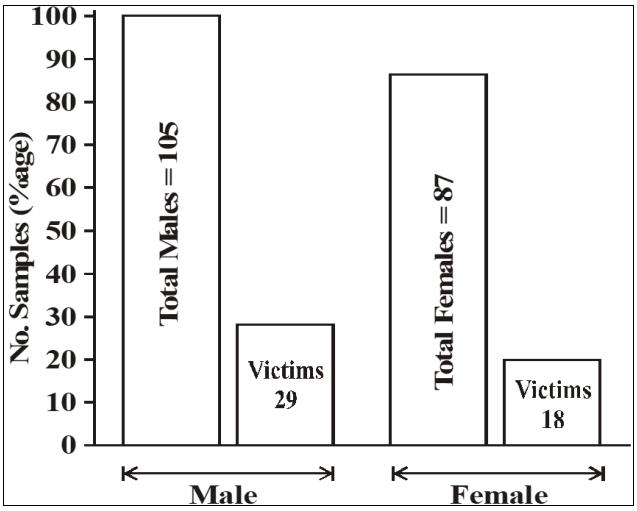

Fig 2: Plot showing of total number of males and females & their victim's along with their percentage

Gender wise result with reference to species

Out of 105 suspected male patients, 29 were found victims of *Plasmodium* infection. In 29 malarial patients, 15(51.72%) were infected by *P. vivax*; 12(41.37%) by *P. falciparum* and 2(6.89%) by mixed species. While other 76 suspected patients were further consulted for diagnosis the disease. In a total of 87 suspected female patients, 18 were suffered from malaria. In 18 malarial patients, 10(55.55%) were suffering from *P. vivax* and 8(44.44%) by *P. falciparum.* Result is ordered in figure 3.

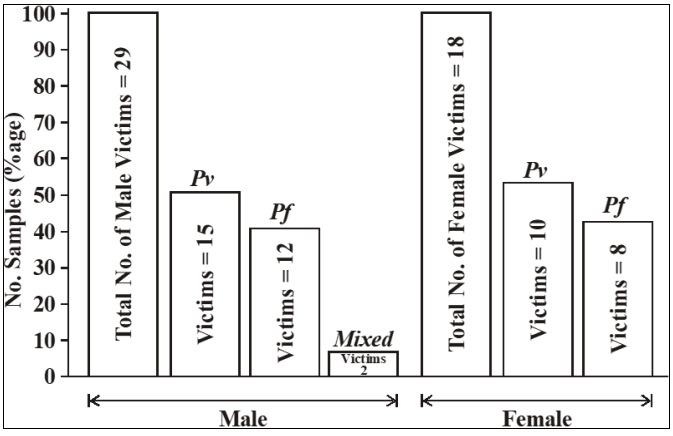

Fig 3: Plot showing of total number of males and females victims, Plasmodium species and their percentages.

Species wise results

In species prevalence comparison, there were more victims of *P. vivax* i.e., 25 (53.19%), and number of *P. falciparum* victims are 20 (42.55%) and the number of mixed species are 2 (4.25%). Result has shown in figure 4.

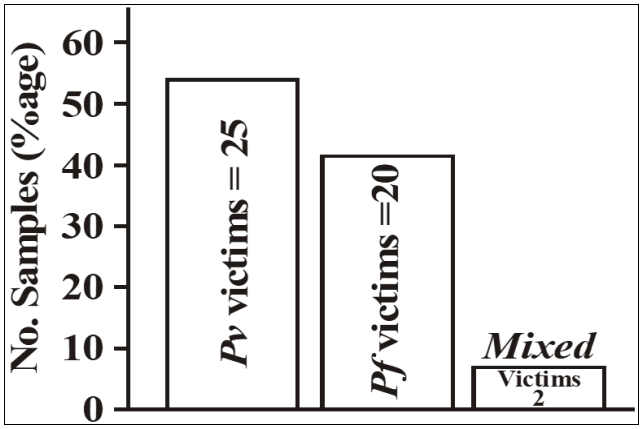

Fig 4: Plot showing of total number of victims of *Plasmodium vivax*, *Plasmodium falciparum* and mixed species along with and their percentages.

Age wise results

The age wise parameter indicated that the age range 21 to 30 years old are more victims consisting of 17 numbers (36.17%) followed by age range 31 to 40 years old consisting of 11 numbers (23.40 %) than the age group 11 to 20 years old with numbers 7 (14.89%). The age group 41 to 50 years old comprises of 5 people with percentage of 10.63 %, while both age groups 1 to 10 and 51 to 60 years old were comprise of 3 numbers respectively having a percentage value of 6.38%. The lowest value was of age group 61-70 comprises of 1 number with percentage 2.12%. The data are also shown by figure 5.

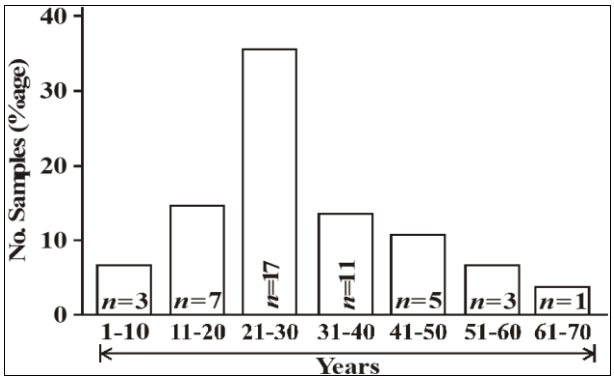

Fig 5: Plot showing results of different age groups along with their percentages.

Seasonal variations affects on *Plasmodium* infection distribution

Seasonal comparison revealed that there was a gradual rise in number of *Plasmodium* victims from March to August and then showed a reduction in severity in the month of September and October. In the month of March 11 blood samples were taken from the patient suffering from high graded fever. Out of these 11, one was found positive and identified as *P. vivax.* In the month of April 12 blood tests were taken. Out of these 12, three were found positive in which two identified as *P. vivax* and one as *P. falciparum.* In the month of May 19 blood tests were performed in which six were found positive, out of which 3 were identified as *P. vivax* and 3 as *P. falciparum.* In the month of June 37 blood samples were screened in which five were positive for *P. vivax* and three were for *P. falciparum.* In the month of July 39 blood tests were examined. Out of these 39, ten were

found positive; the number of *P. vivax* victims was five and for *P. falciparum* was also five, while in the month of August, the number of victims were high. A total of 52 blood samples were screened, out of which 15 samples were detected positive, in which seven were *P. vivax*, six were *P. falciparum* and two were mixed. In September, blood samples were collected from 16 people suffering from high graded fever. Out of these 16, 4 were identified as positive; two belonged to *P. falciparum* and two to *P. vivax*. Six tests were performed in October, but all were found negative for *Plasmodium*. Data is also ordered in tabular form in table 1.

Table 1: Seasonal variations effect on malaria distribution

Season	Month	No. of Sample	*Pv*	*Pf*	Mixed	Total
Spring with moderate temp.	March	11	1	0	0	1
	April	12	2	1	0	3
	May	19	3	3	0	6
Summer with Extreme temp. & Monsoon	June	37	5	3	0	8
	July	39	5	5	0	10
	August	52	7	6	2	15
Hot & Post Monsoon Season	September	16	2	2	0	4
	October	6	0	0	0	0
	Total	192	25	20	2	47

Discussion

Plasmodium infection as health hazard in Pakistan

Pakistan is located in tropical climate and larger part of populace is confined to agriculture and lives in rustic region. The polluted and improper drainage system is a major source of malaria. ICTs are fast, simple to perform and decipher, save time and exertion, and contrast positively with other parasitological tests as a point of-care detection strategy. In rural areas, still microscopy method is used to diagnose malaria. The review of history revealed that *Plasmodium* infection (malaria) always remained the major cause of fatality and the severity and lead to death in the whole world. According to World Health Organization 2017 report, 219 million *Plasmodium* infected cases reported all around the world, contrasted with 214 million cases in 2015 and 239 million cases reported in 2010 while 435000 deaths occurred in 2017 contrasted with 451000 deaths in 2016 and 607000 in 2010. This report also revealed that eighty two percent of *P. vivax* victims residing in five countries, including Pakistan [12].

Comparison with previous research conducted in district Multan

The current research was completed in Multan district where the prevalence of *Plasmodium* infection is higher as compared to prior research conducted at Multan wherein out of 252 high graded fever patients were checked for either the prevalence of *P. falciparum* or *P. vivax*. The result showed that the *P. vivax* had more prevalence with the result 8 (3.17%) as compared to *P. falciparum* with the result 3 (1.19%) [13]. While in this research, out of 192 high graded fever patients, 25 (13.02%) suffered from *P. vivax;* 20 (10.41%) suffered from *P. falciparum* and 2 (1.04%) suffered from mixed species. The result showed that *P. vivax* had more prevalence than *P. falciparum* and mixed species has existed in the region. The occurrence of *P. species* was similar as a past study conducted in Thai-Myanmar border [14]. The higher rate of prevalence of *Plasmodium* types in this research would be because of an increase in population and pollution including stagnant water in this area.

Gender wise comparison

In gender wise data, out of 105 males 29 (27.61 %) were more victims of *Plasmodium* infection than 18 females out of 87 (20.68%). The high number of males as compared to females in this research can be satisfied with the way that males are easily victims of Plasmodium infection on account of their work nature as they work in the fields, garden, filthy places, tyre shops and marshy areas etc. A previous examination in Quetta, Pakistan also showed the large number of males as compared to females with high graded fever [15]. The results of this study also agreeing with another examination in Larkana district of Sindh, Pakistan, which also demonstrated the higher number of *Plasmodium* infection in males than females [6].

Gender wise comparison with reference to species

In gender wise comparison with respect to species, *P. vivax* had more prevalent in males 15 (51.72 %) as well as in females 10 (55.55%) as compared to *P. falciparum* with a number of 12 in males (41.37%) and 8 in females (44.44%). Past examinations taken in district Mansehra and Abbottabad of Province KPK, Pakistan showed a greater number of *P. vivax* infection than *P. falciparum* [16]. *P. vivax* is largely spread in moderate climates and had more commonality as compared to *P. falciparum*. It is observed that *P. vivax* is more capable to develop game tocytemia when the temperature is moderate [17]. Moreover, global environmental changes might be the strongest source of the epidemiology of *P. vivax* in this region [18]. Past observations indicated that mixed infections of *P. vivax* and *P. falciparum* also occurred [19, 20, 21]. It is concluded that *P. malariae* and *P. ovale* were not found in this research.

Age wise comparison

Age wise comparison revealed that, all the ages were victims of Plasmodium infection, but the age range 21 to 30 years old was more victims of Plasmodium infection (36.17%). These observations are comparable to the prior research with the percentage of (30.47%) of the age group of 21 to 30 years [22]. We can say that the age group comprises of 21 to 30 years is the age of art and work. On the account of their working activities they have more chances to bite by mosquitoes as they work and stay outside.

Seasonal comparison

Here we observed a gradual rise in *Plasmodium* infection from the month of March to August. The highest number observed in August 15 (31.91%) while least number was noted during the month of March 1(2.12%). It is already

reported that the gradual increase in temperature was favoring the development and multiplication of *Plasmodium* species [23].

In addition, we noted continuous rise in *Plasmodium* infection from March to August. The previous study also revealed that the steady rise in temperature and rainy season promoting the large reproduction of *Plasmodium* species in the month of July and August [19, 24]. Hence, rise in temperature and rainy season in the month of July and August would be the major cause of *Plasmodium* infection in Multan district.

Conclusion

The prevalence of *P. vivax* was observed at a level that could be a great hazard to public health. The observations of this research will be useful to initiate a quick anti-malaria infection campaign to reduce and counteract the disease in Multan district.

Future work

In developing countries like Pakistan financial condition, access to health care centers and social features varies among areas and populaces leading to the multiplicity of *Plasmodium* infection around there. The control and annihilation of *Plasmodium* infection, requests a functional methodology. Currently we have a number of best quality devices as well as insecticide spraying and properly used of bed nets help to resist the transmission of the disease by means of the mosquito vector (*Plasmodium*).I realize we can overcome *Plasmodium* infection, if all the countries take responsibilities to eradicate this epidemic and help and support others to overcome this misery. This study will be very supportive in future to initiate prompt malaria control program to control this epidemic. This research also gives stress on early diagnosis and prompt treatment. This study also demands for thorough and broad Public inculcation to increase public awareness about malaria, its treatments, control and prevention.

Abbreviation

WHO: World Health Organization
Pf: *Plasmodium Falciparum*
Pv: *Plasmodium vivax*
ICT: Immuno chromatographic Technique;
EDTA: Ethylenediamin etetraacetic acid
HRP: Histidine Rich Protein;
LDH: Lactate Dehydrogenase

Availability of supporting data

All data generated or analyzed during this study are included in this published article.

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