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Prevalence of *Plasmodium Falciparum* and *Plasmodium Vivax* in Choti Zareen, Dera Ghazi Khan, Pakistan

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ABSTRACT

Plasmodium is and endoparasite of human, which belongs to class apicomplexan of Protista. There are many species of this parasite, but here we study about two, which are majorly occurring in Pakistan. Our research area for study was Choti Zareen, D.G Khan, Pakistan. We study the different groups of peoples on the bases of Sex/ Gender, Age, Education, and Socio-economic status. The ratio of malarial disease was higher in females than males, in early age children's and aged peoples than juvenile, in uneducated than educated peoples and in socio-economic peoples and poverty's ones. In this study, 200 human blood samples were collected from Choti Zareen, Dera Ghazi Khan to determine the prevalence of Plasmodium falciparum and Plasmodium vivax. The overall malaria prevalence was found to be 25.5%, with P. vivax (90.1%) being more prevalent than P. falciparum (52.9%). Age-wise distribution revealed the highest prevalence (37.5%) in the 51–60 years group and the lowest (16.6%) in the 61–70 years group. Males showed a higher infection rate (32.2%) compared to females (20%). A negative correlation was observed between educational level and prevalence: uneducated individuals had a higher rate (28.5%) than those with secondary or higher education (18.42%). Socioeconomic status also played a role, with the highest prevalence in the lower class (32.1%) and the lowest in the upper class (17.2%). These findings suggest that age, sex, education, and socioeconomic factors significantly influence malaria transmission in the region, highlighting the need for targeted awareness and control measures.

INTRODUCTION

Plasmodium is one of the groups of apicomplexan parasites responsible for causing malaria in a wide range of vertebrates including humans [1]. Malarial infection is a hazardous sickness that takes lives of about one million people each year and influences the same number of people, living in 109 countries in Asia, Africa, and Latin America [2]. Plasmodium is one of the eukaryotes, it has all the morphological features of a eukaryotic cell. All the structures or organelles present in a eukaryotic cell are part of a Plasmodium cell, such as nucleus, endoplasmic reticulum, Golgi complex and mitochondria are present in it. In addition to these organelles there are some other organelles of Plasmodium which are unique characteristics of apicomplexans and help them in their parasitic mode of life such as apical complex and micronemes [3]. There are five different polymorphs of Plasmodium species present during their life history in different hosts.

Trophozoites are the feeding stage of Plasmodium species which may be observed in peripheral blood of man when it is in growing stage but once it matures it penetrates towards the deeper arteries and is observed in peripheral blood except in case of heavy infection. Its cytoplasm appears coma shaped, and chromatin may be

seen as a single or double dot P. vivax trophozoites usually have irregular amoeboid appearance [4]. two types of gametocytes are formed inside the mosquito host, microgametocytes and macrogametocytes. The morphological features (shape, size, and structure) of gametocytes vary in different species of Plasmodium. Microgametocytes have a larger more diffused nucleus while macrogametocytes have darkly stained cytoplasm due to presence of large number of ribosomes. These ribosomes are involved in extensive protein synthesis needed for fertilization. In mosquito host microgametocytes are produced by the Ex-flagellation of merozoites usually measures about 15-25 micrometers in length. While on the other hand macrogametocytes are somewhat rounded in shape and measures about 7- 14 micrometers [5]. After fertilization of microgametocytes and macrogametocytes a motile zygote is formed which is known as ookinete. It is enclosed by a 2-layered envelope, consisting of an outer rough and inner smooth layer [6]. Oocysts are formed by the migration and penetration of ookinete into the gut wall of mosquito and measure about more than 50 micrometers in diameter. The oocysts generate several thin and elongated sporozoites which measure about 15 micrometers in length, and these ultimately reach inside the salivary glands of mosquitoes. It has a thick double membrane, external membrane is corrugated, and inner is stouter [7,8].

Mode of Transmission

Plasmodium species are transmitted to the vertebrate host by the bite of Female Anopheles mosquitoes. Out of 480 species of Anopheles, just around 50 species can transmit malaria [9,10,11].

The Female mosquitoes suck the blood of vertebrate's host for the nourishment of their eggs. At the point when a mosquito obtains blood meal from their host, it transfers the gametocytes to the blood of their host. These gametocytes undergo sexual development in the gut of mosquito and converted into sporozoites.

Pathology

There are numerous species of Plasmodium which spread malaria fever in the people of the world, however in Pakistan this malady is caused because of three species *P. vivax, P. falciparum* and *P. malariae. P. vivax* causes malaria in which the eruptions repeat at regular intervals. *P. falciparum* causes destructive type of malaria in humans [12]. This species is cosmopolitan in distribution, but major habitats are tropical and subtropical areas of world.

It was probably the worst enemy of humankind, particularly in Africa. *P. malariae* causes malaria fever with the eruptions that repeat periodically. This specie is worldwide in distribution. *P. ovale* is rarest among all. It is basically tropical in dispersion. This type of Plasmodium is most of the time lethal. There are four kinds of Plasmodium infections brought about by these four Plasmodium species [13].

Diagnosis

Microscopic examination of Plasmodium species by thick and thin blood smear is one of the most common diagnostic tests of malaria. Thick smear examination is rarely used. Sometimes, it becomes necessary to administer anti-malarial drugs as soon as the clinical diagnosis has been made and in these cases blood slides should be taken by the physician himself and sent to the laboratory for examination [14]. If parasites are numerous the species can easily be identified in a well stained film. But species identification becomes difficult when only a few rings are encountered. In such cases, however, the blood should be examined a few hours after correct diagnosis.

Treatment

Various medications have been synthesized to control malarial infection caused by plasmodium in vertebral hosts, especially in human beings. The synthetic drug Quinine was utilized as a most ideal remedy of malarial medication from the seventeenth century until far reaching obstruction rose in the mid twentieth century [15].

Protection from quinine initiated the advancement of a wide scope of malarial drugs in ongoing period, for example, Chloroquine, Proguanil, Sulfadooxine, Atovaquone, and Artemisinin Chloroquine. In all the cases, parasites resistance to a given medication developed inside a couple of many years of the medication organization [16]. To defeat this circumstance, malarial medications are every now and again utilized in artemisinin mix treatments at present the best quality level for treatment [17]. Generally malarial medications focus on the formative phases of Plasmodium parasites that dwell inside the vertebrate erythrocytes, as these are the phases which generate general reason ailment [18].

Epidemiology and Control

There were 311 million clinical instances of P. falciparum malaria worldwide in 2002 [19]. Pakistan had been declared a country with moderate malaria pervasiveness and generally entrenched control programs. This infection is assessed to cause at any rate 50,000 passings every year [20]. It is a noteworthy medical problem worldwide particularly in the number of inhabitants in the tropical and subtropical territories. Also, there is a dynamic relocation of contaminated human population the nation over, which is a noteworthy contributing variable for the spread of infection and scattering of medication safe malarial sickness parasites [21]. Malarial infection happens essentially in the territories with poor access to an officially delicate medicinal services framework.

There are types of humans are under most elevated hazards; one is pregnant women and other are children under 5 years. As indicated by the World Health Organization's 2010 roughly, 781,000 individuals passed on due to this sickness in 2009. Numerous deaths happened in Africa, Bangladesh, and Ethiopia because of the malarial sickness and most of the cases were kids. Poor financial improvement is viewed as the real reason for the wide dispersion of malaria [22].

About 3.2 billion individuals were recorded at the danger of malaria fever, and there were 212 million malaria cases around the world, representing almost 4, 29,000 passings, of which 92% were in WHO African locale, 6% were in WHO South-East Asian regions, and 2% were in WHO Eastern Mediterranean area. The highest

weightage of prevalence was recorded in the populations that were poor and malnourished. Transient workers venturing out to the endemic regions, children under5 years and pregnant ladies are at high-chance gatherings that are influenced by the high weight of malarial sickness [23, 24].

Control of Larvae

Mosquito larva is controlled by using diesel oil [25], Oil layer spread on the surface of water causes deficiency of oxygen, so it becomes difficult for larvae to respire 17 and death results. As well as oil is also toxic and proves fatal for larvae. Spray insecticide is also effective for controlling larvae, but it is not a good method for many beneficial species of insects and fishes. Biological control is a safer method as compared to chemical control.

For this purpose, predatory fishes such as Gambusia and Toxorhynchites larvae perform a great role in controlling the mosquitoes' larvae. Similarly, a specie of nematodes Romanomenamis petersoni and a protozoan specie Nosemaalgaree are used for this purpose. Some bacterial species such as Bacillus thuringiensis and Bacillus sphaericus have effective control on adult mosquitoes` growth [26].

Apart from chemical and biological control some precautionary measures also reduce the growth rate of mosquitoes` larvae such as:

- Eradicating the swampy areas by filling or draining which are the habitat of mosquitoes' larvae.
- By removing man-made reservoir of water such as tires, water pots, cans bucket etc.
- Irrigation water should be used properly.
- Use of insect growth regulators such as dimilin prevents the developmental stages to mature.

Control of Adult Mosquitoes

Adult mosquitoes are controlled by using aerial sprays. Long residual action spray such as DDT with specific dosage is effective in buildings or houses. For personal protection mosquito repellents lotion should be applied on the body as well as doors and windows should be screened and bed nets should be used. Sound traps are also used for this purpose. These are the devices which produce a specific sound so that mosquitoes are attracted towards them and killed. Birds such as purple martin feed upon adult mosquitoes and perform an important role in controlling it [27, 28].

Objectives of the Research

1. To explore the overall prevalence of Plasmodium species in Choti Zareen, Dera Ghazi Khan.
2. To estimate the relationship between age and seroprevalence of *P. falciparum* and *P. vivax* in Choti Zareen, Dera Ghazi Khan.
3. To study the relationship between sex and seroprevalence of *P. falciparum* and *P. vivax* in Choti Zareen, Dera Ghazi Khan.
4. To understand the relationship between educational status and Seroprevalence of *P. falciparum* and *P. vivax* in Choti Zareen, Dera Ghazi Khan.
5. To elaborate the correlation between socioeconomic status and seroprevalence of *P. falciparum* and *P. vivax* in Choti Zareen, Dera Ghazi Khan.

MATERIALS AND METHODS

This research was designed to assess the prevalence of *Plasmodium falciparum* and *Plasmodium vivax* in the humans of Choti Zareen, Dera Ghazi Khan. There are 200 blood samples were collected from humans in the study area. The Age, Sex/ Gender, Educational Status, and Socioeconomic Status of the subjects were recorded. Combo Rapid Diagnostic kits were used for the finding of Plasmodium species.

This test identified the antibodies present in the blood serum, plasma or entire blood that were developed in the subject due to *P. falciparum* and *P. vivax*. This test was a viable technique for diagnosing malarias. It provides an easier way for the separation of infection with *P. falciparum* and *P. vivax* and could be performed in 15-20 minutes without the utilization of microscope facility. Appearance of Pf or Pvlines or both the lines one after another showed the positive results. An inner control (C line) indicated the positive and agative results similarly.

Principle

During this study, Combo Rapid Test was performed which is a double antigen lateral flow chromatographic immunoassay. When an appropriate amount of test samples is dispensed into well of the test cassette, it migrates across the cassette. Antibodies (IgG, IgM, and IgA) bound to Pf or Pvconjugates respectively. The immunocomplexes are then captured on the membrane. MSP antigens formed a burgundy-colored Pf line (Pf positive result) and /or Pv line (Pv positive result). Absence of any test lines suggests negative results. An internal control (C line) exhibits positive and negative lines equally.

Method

The test was performed by the strategies explained by the producer leaflet. First, packets were opened at the indent, kit was expelled and set it on a spotless surface. At that point every device was named with a sample ID number. Blood samples were collected by utilizing disinfected needles. Slender cylinder was topped with blood off to 20 µL. The whole measure of sample was put into well of the kit. Promptly, two drops of test diluent (60-80 µL) were added to the sample well situated vertically. Results were recorded in the following 15-20 minutes. After that results were elaborated through Chi-Square test.

RESULTS AND DISCUSSION

Overall prevalence of *P. falciparum* and *P. vivax* in humans of Choti Zareen, Dera Ghazi Khan, Pakistan. In this research 200 samples were collected from the humans of Choti Zareen and Dera Ghazi Khan to observe the overall prevalence. It was observed that the overall prevalence of *P. falciparum* and *P. vivax* was 25.5%. The prevalence of *P. vivax* (90.1%) was comparatively greater as compared to *P. falciparum* (52.9%). Results are illustrated in Figures.

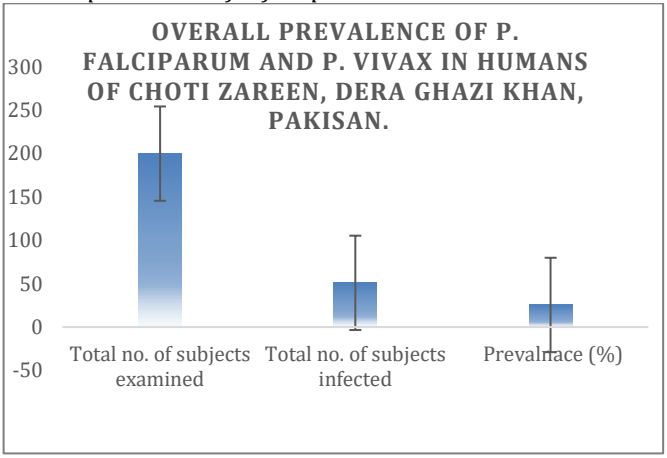
The Overall prevalence of *P. falciparum* and *P. vivax* in humans of Choti Zareen, Dera Ghazi Khan, Pakistan.

During our research 200 samples were collected from Choti Zareen and Dera Ghazi Khan to observe the overall prevalence of *Plasmodium* species.

The prevalence of *P. vivax* (90.1%) was comparatively greater as compared to *P. falciparum* (52.9%). Results are illustrated in Figures. I, II and III.

Figure I

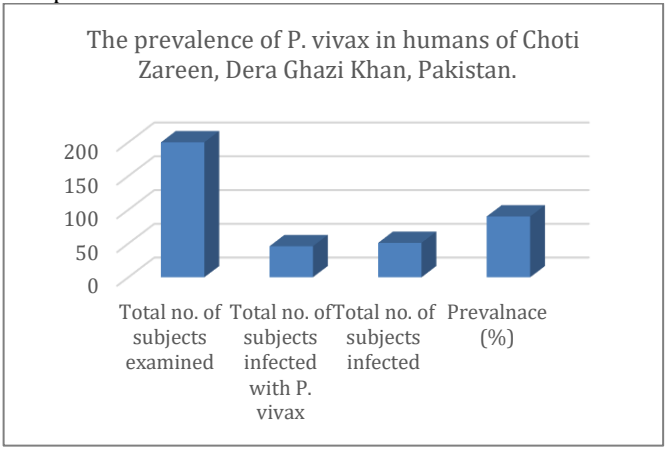
Overall prevalence of P. falciparum and P. vivax



The prevalence of *P. vivax* in humans of Choti Zareen, Dera Ghazi Khan, Pakistan.

Figure II

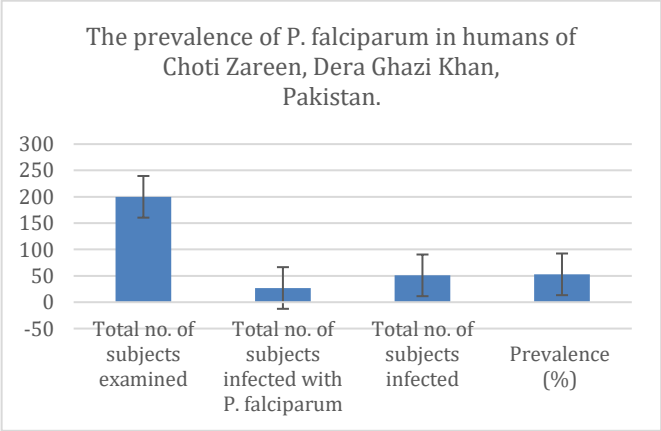
The prevalence of *P. vivax*



The prevalence of *P. falciparum* in humans of Choti Zareen, Dera Ghazi Khan, Pakistan.

Figure III

The prevalence of *P. falciparum*

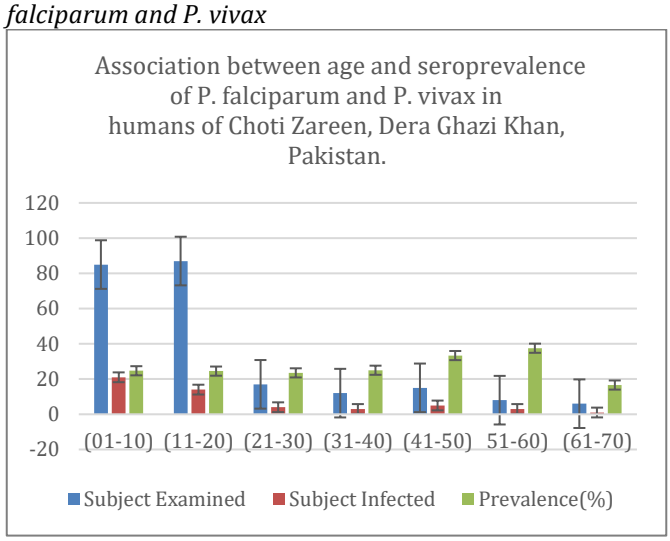


Association between age and seroprevalence of *P. falciparum and P. vivax* in humans of Choti Zareen, Dera Ghazi Khan, Pakistan.

Based on the age factor the data was divided into eight different groups. The results of research prove the prevalence of *plasmodium* species in different age groups was 24.7% in 1- 10 years age group, 24.5% in 11-20 years age group, 23.5%, in 21-30years age group, 25.0% in 31-40 years age group, 33.33% in 41-50 years age group, 37.5% in 51-60 years age group and 16.6% in 61-70 years age group. Highest rate of prevalence (37.5%) was observed in the age group 51-60 years and lowest prevalence rate (23.5%) was observed in the age group of 21-30 years. Results are exhibited in Graph IV.

Figure IV

Association between age and Seroprevalence of P. falciparum and P. vivax

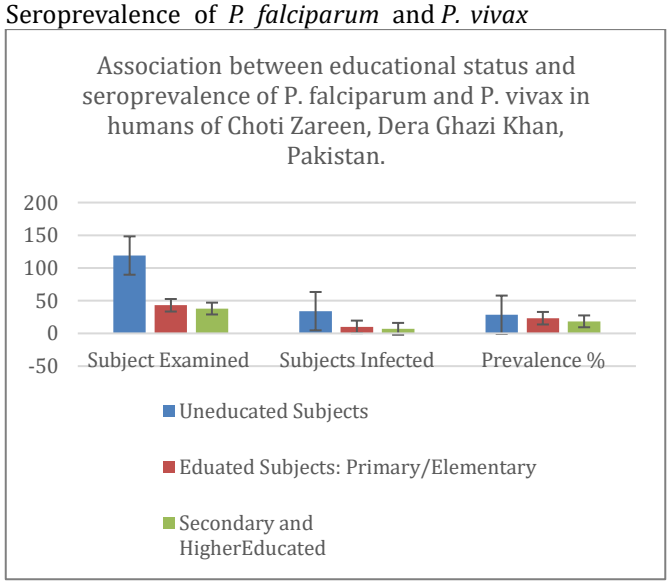


Association between Educational Status and Seroprevalence of *P. falciparum* and *P. vivax in* humans of Choti Zareen, Dera Ghazi Khan, Pakistan.

Because of educational status, the data was divided into two major categories, uneducatedand educated subjects. As illustrated in Graph V the prevalence of *P. falciparum* and *P. vivax* (28.5%) was greater in uneducated subjects than in Primary/Elementary level educated subjects (23.25%) and secondary and higher educated subjects (18.42).

Figure V

Association between Educational Status and Seroprevalence of *P. falciparum* and *P. vivax*

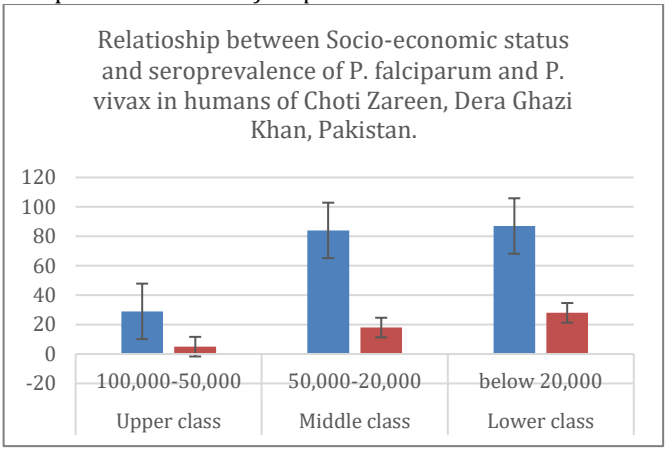


Relationship between Socio-economic Status and Seroprevalence of *P. falciparum* and *P. vivax* in Humans in of Choti Zareen, Dera Ghazi Khan Pakistan

In the present study data was divided into three socioeconomic categories and it was observed that the prevalence in upper class was 17.2%, 21.4% in middle class and lowerclass had 32.1% prevalence. Results are exhibited in Graph VI. According to Chi-Square analysis it was inferred that, the relationship between Socio-economic status and prevalenceof Plasmodium species was statistically nonsignificant (p>0.05).

Figure VI

Relationship between Socio-economic Status and Seroprevalence of *P. falciparum* and *P. vivax*

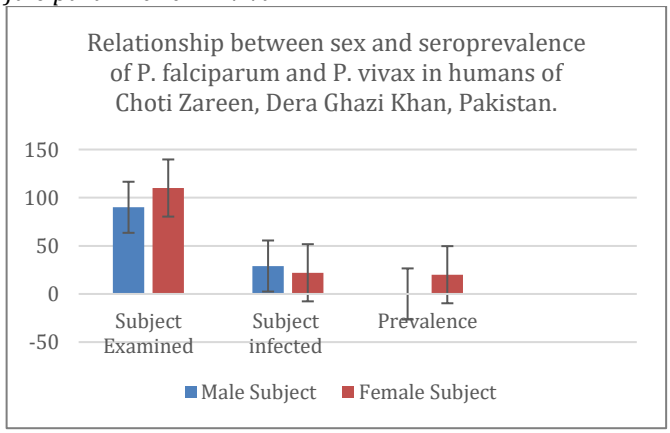


Relationship between Sex and Seroprevalence of P. falciparum and P. vivax in humans of Choti Zareen, Dera Ghazi Khan, Pakistan.

Data was divided in two groups based on sex, males, and females. Results of the present study showed that the overall prevalence of *P. falciparum* and *P. vivax* was greater in males (32.22%) than females (20.0%). Results are presented in Graph VII.

Figure VII

Relationship between Sex and Seroprevalence of *P. falciparum* and *P. vivax*



DISCUSSION

The Overall prevalence of *P. falciparum* and *P. vivax* in humans of Choti Zareen, Dera Ghazi Khan, Pakistan.

According to the results of research, the overall prevalence of *P. falciparum* and *P. vivax* was 25.5%. The prevalence of *P. vivax* was 90.1% and *P. falciparum* was 52.9%. Various researchers have worked on the prevalence of *Plasmodium* species in various parts of the world including Pakistan [29,30]. The recorded the Seroprevalence of *P. falciparum* (76%) and *P. vivax* (43%) from all provinces of Pakistan including Islamabad.

Overall prevalence (79.6%) for *P. vivax* and (20.3%) for *P. falciparum* in the humans of district Rajanpur in South-Western Pakistan. The prevalence of *P. vivax* (5.78%) and *P. falciparum* (1.08%) in district Buner. The prevalence of malarial infection in Sarbaz, Sistan and Baluchistan provinces. The results showed that 90 % of isolated species were *P. vivax,* 7.8% *P. falciparum* and 2.2 % were *P. malariae* [31, 32]. Overall Seroprevalence of *P. falciparum* (69.5%) and that of *P. vivax* (30.2%) in district Ziarat and Sanjavi, *P. falciparum* (52.8%) and *P. vivax* (47.15%) inthe Larkana district Sindh, overall prevalence of *P. vivax* (92.56%) and *P. falciparum* (7.44 %) in district Mardan Pakistan [33].

Results of the present study are notin line with the results reported by [34, 35]. The higher prevalence of *Plasmodium* species in many areas of world might be responsible for the prevailing environmental conditions such as slums, high rainfall, defaulted sewerage system, stagnant water, improper dumping of garbage, relative humidity, much surface water, septic ditches, and stagnant pools which act as vector breeding sites [36]. It was recorded that the prevalence of *Plasmodium* species was higher in rainy season than in dry season due to larger amount of standing water areas which act as breeding sites for mosquitos' larvae [37,38]. Higher prevalence of malaria might be due to lack of knowledge and lack of appropriatepreventive practices for using ITNs [39].

Association between age and seroprevalence of *P. falciparum* and *P. vivax* in humans of Choti Zareen, Dera Ghazi Khan, Pakistan.

The results of the Present study revealed that the prevalence of *P. falciparum* and *P. vivax* in different age groups was 24.7% in 1- 10 years age group, 24.5% in 11-20 years age group, 23.5%, in 21-30 years age group, 25.0% in 31-40 years age group, 33.33% in 41-50 years age group, 37.5% in 51-60 years age group and 16.6% in 61-70 years age group (p>0.05). Research has been conducted on the same parameter in different parts of the world. The highest prevalence of 3.6% in the age group of greater than 15 years old persons [40,41,42].

Increased stepwise from 6.1% in children less than ten years to 36.5 % in subjects greater than fifty years. Among 6.8 % positive cases highest number of cases (47.41%) were observed in the age group of 0-20 years in district Mardan, Pakistan [43]. There is highest parasite density of 8080 µl in 0-3 years of age than in 4-6 years with 46000 µl in parts of port Harcourt metropolis [44, 45].

Relationship between Sex and Seroprevalence of *P. falciparum* and *P. vivax* in humans of Choti Zareen, Dera Ghazi Khan, Pakistan.

Results of our research showed that the overall

prevalence of *P. falciparum* and *P. vivax* was (32.22%) in males and female had (20.0%) prevalence (p>0.05). The females had 50 % more chances to get malarial infections than males. The seroprevalence of *Plasmodium* species was higher among males (7.10%) as compared to females (6.52%) in general population. The Seroprevalence of *Plasmodium* in males (65%) was higher as compared to females (35%) in Pakistan. Many controlling factors are responsible for higher seroprevalence of *Plasmodium* species in males as compared to females. It is usually related with the sociological and physiological factors and specifically hormonal in origin.

Association between Educational Status and Seroprevalence of *P. falciparum* and *P. vivax* in humans of Choti Zareen, Dera Ghazi Khan, Pakistan.
It was seen in the present examination that the seroprevalence of *P. falciparum* and *P. vivax* was (28.5%) in uneducated subjects and it was (23.25%) in primary/elementary level subjects and (18.42%) in Secondary and highly educated subjects (p<0.05). Different researchers had worked on the relationship of educational status.

Relationship between Socio-economic status and seroprevalence of *P. falciparum* and *P. vivax* in humans of Choti Zareen, Dera Ghazi Khan, Pakistan.
In the present study it was observed that the seroprevalence of *P. falciparum* and *P. vivax* in upper class was 17.2%, 21.4% in middle class and lower class had 32.1% (p>0.05). Many reports from different parts of the world including Pakistan have revealed that there is a close association between prevalence of *P. falciparum* and *P. vivax* to the socio-economic status.

The seroprevalence of malaria was higher in rural children than urban children, as well as poor children compared to rich. There is higher prevalence of malaria in poorly developed provinces of Pakistan like Sindh, Baluchistan, and FATA than more developed province, Punjab. Higher percentage in lower class highlights the fact that malaria is adisease of poverty.

Among other factors, life in poor areas with slum like conditions is also a contributor for increased malaria transmission. There is a negative effect between the family household head`s age and malaria prevalence. Educational level of head of household was not significantly related with malaria. But the profession of household had a significant effect on malaria.

CONCLUSION

The results of our study indicates that, the seroprevalence of *P. falciparum* and *P. vivax* observed in the study area is 25.5%. Significant association was observed between age, sex, educational and socioeconomic status to the distribution of *Plasmodium* species.

Future Recommendation

1. There is a requirement for population-based study to observe the Seroprevalence of *P. falciparum* and *P. vivax* infections in research area.
2. There is a need for future investigations about spatial dispersion of *Plasmodium* species and related factors for causing malaria fever in the study area.
3. There is a need to educate the residents of Choti, Zareen, Dera Ghazi Khan to use precautionary measures.

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