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Research Article

Prevalence of Malaria and its Association with ABO Blood Groups in District Battagram

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Abstract: Malaria is a protozoan disease caused by the Plasmodium parasite and is moderately endemic in Pakistan. This study examined the correlation among malaria prevalence, ABO blood groups, and environmental risk factors in District Battagram. A total of 200 participants were screened for malaria by blood smear microscopy, and an agglutination test was performed to determine the blood group. The malarial prevalence was found to be 11.5%, reporting *Plasmodium vivax* 10.5% (n = 21) and *falciparum* 1% (n = 2). The highest number of malaria cases were in people with blood group B (10.35%), followed by blood group A (4.27%), and AB (0.07%), while no case was reported in participants having blood group O. Among the risk factors, sleeping outdoors and not having a past malaria history were two of the risk factors that were significantly associated with malaria positivity ($p < 0.05$). Other variables such as age, gender, closeness to stagnant water, and utilization of mosquito nets showed the prevalence but did not demonstrate a significant statistical correlation. It is suggested that blood group and malaria susceptibility can inform the targeted public health strategies for malaria prevention and control.

Keywords: Malaria, ABO Blood Group, *Plasmodium,* Epidemiology, Risk Factors.

1. INTRODUCTION

Malaria is a protozoan disease cause by *Plasmodium* and transmitted via female Anopheles mosquitos. To date, 120 species of *Plasmodium* are known so far; 5 species of the *Plasmodium* cause malaria: *P. malariae P. vivax, P. falciparum, P. ovale*, and *P. knowlesi*. *P. falciparum* causes high mortality, constituting over 99% of global malaria-related fatalities. While *P. vivax* has traditionally been considered a causative agent of mild malaria; however, evidence indicates its potential to induce severe illness [1]. Similarly*, P. malariae P. ovales*, and *P. knowlesi* are known to cause malaria in humans. In a study, 128 malaria patients were studied in Parma Italy, 8 *Plasmodium ovale curtisi* and 4 *Plasmodium ovale wallikeri* infections were discovered, but no *P. knowlesi* infections were found [2]. Malaria affects about half of the global population and is endemic in tropical and subtropical regions, which encompass, Eastern Mediterranean, South-East Asia, Western Pacific, the Americas, and, Sub-Saharan Africa [3]. 2.38 million cases per annum were reported in India from 1990 and 2000 which have been declined by 91% from 2011-2022 due to advancement in treatment and vector control strategies. The decline can be attributed to significant interventions, including the Intensified Malaria Control Project (IMCP), Enhanced Malaria Control Project (EMCP), artemisinin-based combination therapy (ACT), bivalent rapid diagnostic tests (RDT-Pf/Pv), and the participation of Accredited Social Health Activists (ASHAs). Holt's models and Autoregressive Integrated Moving Average

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(ARIMA) forecast zero indigenous cases in India by 2027-2028. The population density, literacy rates, health facilities, and accessibility to healthcare facilities have reported to greatly impact malaria incidence and outcome [4]. The case fatality rate of malaria in Sub-Saharan Africa constituted 94% of total deaths. Children under five years old represent the highest proportion of severe disease burden, constituting 67% of global mortality [5]. Pakistan has a high malaria incidence, with over 670,000 cases and 3159 deaths reported cases in 2013 [6].

According to the WHO report, Pakistan has ~700,000 cases of malaria in year 2019 [7]. In 2024, WHO published its yearly report on global malaria control and elimination trends [8]. Despite advancements in malaria prevention, the data indicates that routine vaccinations, there were still over 11 million more cases in 2023 compared to 2022. WHO predictions indicate that there were 263 million cases of malaria globally in 2023 (95% CI 238 million to 294 million). According to the WHO report published in 2023, by 2025, the Global Technical Strategy for Malaria 2016-2030 aims to reduce the malaria fatality rate by 75% [5].

Malaria is highly endemic in the region and a study from neighboring district "Shangla" in 2017 reported *Plasmodium* in 13.9% (n = 87) of the suspected cases. The percentage of positive cases was greater in males 65.24% while females had a positive rate of 34.76%. In the study sample, the highest prevalence of malaria was found in Group B 51.34%, followed by Group A 33.68%. In Pakistan *P. falciparum* and *P. vivax* are common [9]. In another study conducted in a neighbor district Mansehra, malaria was found in 154 cases 1999-2004, which includes 114 males and 46 females. There were 142 cases of *P. vivax* and 12 of *P. falciparum* [10].

The clinical symptoms of malaria vary significantly between children and adults. Patients with uncomplicated malaria may show modest symptoms such as fever and chills. Severe malaria, on the other hand, can cause life-threatening complications such as severe anemia, acute respiratory distress syndrome, hypoglycemia, shock, metabolic acidosis, acute renal injury, and cerebral malaria. This diversity emphasizes the importance of quick identification and treatment, particularly in youngsters, who are more likely to experience poor disease consequences. Understanding these clinical manifestations is critical for malaria management in endemic areas [11].

The antigens of human ABO blood types exhibit distinct phenotypes and glycoconjugate structures on the surface of red blood cells, influencing both physiology and disease. Researchers have been studying the association between blood type and disease since the early 1900s. It was found that antibodies and antigens are inherited traits. Some blood types lack antigens, leading to debate over the link between blood group and susceptibility to specific diseases [12]. In 1967, Athreya and Coriell [13] conducted an early review, suggesting a possible association between ABO blood groups and malaria. Later, Rowe *et al.* [14] demonstrated that *P. falciparum* rosetting, a virulence factor linked to severe malaria, occurs at lower levels in blood group O erythrocytes compared to blood groups A, B, and AB. A study proposed that blood group O provides resistance to severe *falciparum* malaria through a mechanism involving reduced rosetting. Malaria remains to be a significant global health challenge, especially in tropical and subtropical areas, causing significant morbidity and mortality. Prevalence of malaria and its association have not been studied in district Battagram. Some of the previous studies in Pakistan have tried to establish correlation between ABO blood groups and malaria susceptibility and severity. However, exact dynamics of the relationship might vary across different population. The specific problem is determining the prevalence of malaria in targeted population with different blood groups and socioeconomic parameters and understanding if certain blood groups are more susceptible or resistant to malaria infection in the study population.

The present research aimed to investigate the statistical relationship among blood group types and the incidence of malaria. Malaria is prevalent in district Battagram due to environmental factors favorable to mosquito breeding. Certain blood groups have been implicated to offer some degree of protection against severe malaria, while Blood groups A and B show greater susceptibility. A few global studies have looked into the relationship between malaria and blood groups, but there is limited data on this association in Pakistan, district Battagram in particular.

2. MATERIALS AND METHODS

2.1. Study Area

The present study was carried out in District Battagram which shares geographical boarders with Kohistan to the North, Mansehra to the East and South East, Torghar to the South, and Shangla to west. It has an elevation of approximately 1038 meters from sea level and located in 34°41'N latitude 73°1'E longitude as shown in Figure 1. The research study was conducted from September 2024 to May 2025 in the District Battagram. The rainfall varies throughout the year, from July to September, with the monsoon season contributing the precipitation, resulting in providing favorable condition for mosquito's reproduction and growth. The average annual rainfall is estimated to be around 800 to 1,000 millimeters. The rainy season provides significant moisture to the area.

2.2. Blood Typing (Agglutination Test) for ABO Blood Group

A total of 200 individuals were randomly selected in District Battagram but the sample classes were not balanced. Finger prick was performed on each participant using aseptic blood lancet. A drop of blood was placed on a clean microscopic glass slide and antisera was added to performed agglutination test followed by documenting of results [15].

2.3. Preparation of Blood Smear for Malarial Parasite (MP) Test

Blood smears were prepared from each blood sample collected from participant in targeted population. Sample collection will be performed via finger pricking, thick and thin smear were made followed by soaking the prepared slides in methanol for chemical fixation. The chemically fixed smear were stained with Giemsa stain and incubated for fifteen minutes. Finally, the sample were washed with clean water with precaution to avoid any damage to smear. The resulting slides was stored in a designated slide box for future microscopic reconfirmation in the college laboratory [16].

2.4. Microscopy

The stained blood smears were examined under a microscope at 100x objective lens. After carefully identifying any *Plasmodium* parasites, the data were recorded in excel sheets. According to Maqsood *et al.* [17] detection of malaria from the microscopic blood smears was 96.82% accurate.

2.5. Data Analysis Using SPSS Software

Data collected via questionnaires were summarized and input into Microsoft Excel 2013. A chi-square test was applied to examine the relationship between test results and associated risk factors utilizing SPSS [15].

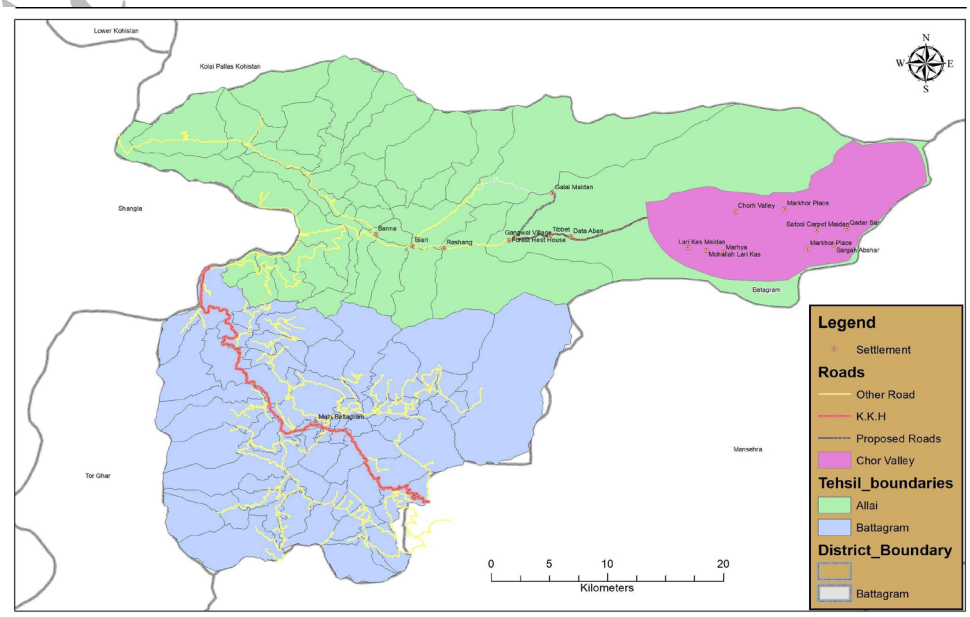

Fig.1. Geographical map of district Battagram.

3. RESULTS AND DISCUSSION

Plasmodium species are usually spread by an arthropods, most often the female Anopheles mosquito [18]. The prevalence rate of malaria in (200) blood sample was 11.5% (n = 23). Among them, 91.3% (n = 21) was *P. vivax* and 8.7% (n = 2) was *P. falciparum*. According to WHO, >1.8 million cases were being reported every year in Pakistan [19]. In 2020, the cases were reduced by 40% compared to 2015 but again soar to over 2 million in 2022 [20].

ABO Blood group is a system for compatibility and ABO antigen association with infection [21]. The result of ABO Blood group showed a percentage prevalence of 10.35% (n = 15) in blood group "B", 4.27% (n = 7) in blood group "A", 0.07% (n = 2) in blood group "AB" while no case was found positive in "O" blood group (Figure 2). The research demonstrates that individuals possessing blood group O may have a comparative protective benefit against malaria infection. This finding supports other studies showing that RBC's of blood group O are less able to clump together, which is a process that helps parasites stick and can cause serious illness. As a result, the lower ability of blood group O to form rosettes may play a vital role in reducing the susceptibility to malaria. Although blood groups A and B have the same percentage exposure to malaria in the studied population, blood group B is observed to be more prevalent. Similar results were reported by Tadesse *et al.* [15] showing higher prevalence in A (40%) and B (34.1) blood group followed by AB (14.3%) and O (5.1%), respectively. Likewise, Panda *et al.* [22] reported that the prevalence of blood group 'B' was significantly higher in patients with severe malaria relative to those with uncomplicated cases ($P < 0.0001$). Regardless of clinical severity, blood group 'B' demonstrated a significant relationship with cerebral malaria ($P < 0.0001$).

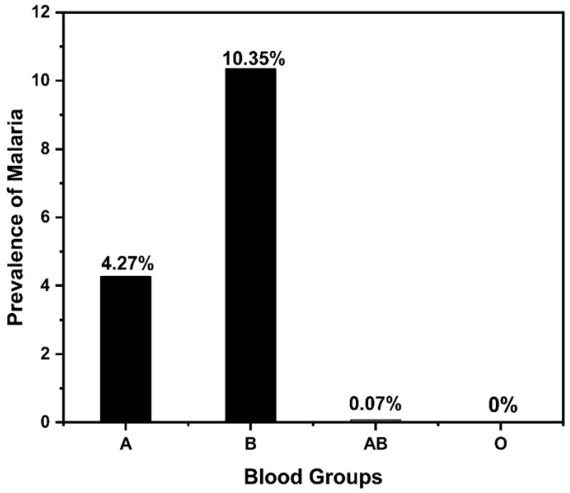

Fig. 2. Percentage prevalence of Malaria in different blood groups.

Many risk factors associated including demographic and epidemiological variables were studied. The study documented the prevalence of malarial is 25.49% in patient who slept outdoor while 6.76% was recorded in patients sleeping indoor. Parameters Like, sleeping area and past malaria history showed a significant relationship ($p < 0.05$) with the presence of malarial parasites in blood, which is mentioned in Table 1. The results indicate that both sleeping area and past malaria history are strongly linked to the infection caused by *Plasmodium*. The finding suggests that both factors may be important in how malaria spreads in the study population. This aligns with previous research that outdoor sleepers and night time activities were prevalent and could substantially increase the risk of malaria. The study documented 42% of the participants sleeping outdoors were at risk of malaria infection in southern Ghana [23]. Studies have demonstrated a 50% decrease in indoor vector density, resulting in a subsequent drop in malaria, due to the screening and windows doors [24]. Most of the positive malaria cases have been seen in homes that don't have window screens. Therefore, windows mesh is very significant for lowering the number of malaria cases in malaria endemic regions [25].

The parameters like patient age, gender, standing water, rice paddies, use of mosquito repellent coils, use of anti-malarial spray, and use of mosquito nets showed trend and act as a risk factor but are found to be non-significant ($p > 0.05$) with the presence of malarial parasites in blood. Higher prevalence was observed in patients over the age of 20 years (10.44%), and the percentage in the patients of the age group less than 20 years was (4.69%). Our study co-relates with the population-based study that identified the prevalence of malaria across several age groups in Colombia, identifying the highest risk age range as 20-30 years [26]. On the other hand, factors including gender, closeness to standing water and rice paddies, and the use of

Table 1. Demographic features, epidemiological variables, and risk factors associated with malaria.

Variables		Prevalence (%)	Significant difference (df)	Chi-square value	P-value
Patient Age	6-10	0.4	3	1.196	0.754
	11-15	1.23			
	15-20	3.06			
	>20	10.44			
Gender	Male	13.3	1	1.797	0.132
	Female	9.36			
Sleeping Area	Outdoor	25.49%	1	13.022	0.001
	Indoor	6.76%			
Standing Water	Yes	19.05	1	0.009	0.561
	No	5.68			
Rice Paddies	Yes	11.76	1	0.089	0.469
	No	11.11			
Past Malarial History	Yes	1.52	1	19.176	<0.001
	No	27			
Symptoms	Chills	3.1	3	4.753	0.191
	Episodic Fever	8.1			
	Anemia	0			
	Other	3.96			
Anti-Malarial spray	Yes	2.52	1	0.388	0.350
	No	26.69			
Mosquito Net	Yes	5.44	1	0.004	0.559
	No	19.65			
Health Facilities	Yes	19.05	1	0.022	0.539
	No	5.76			
Family member	1-5	3.1	2	1.197	0.550
	6-10	8.1			
	>10	4.48			
Climate	Dry	32.8	1	0.371	0.394
	Rainy	1.05			
Blood Group	A	4.27	3	15.151	0.002
	B	10.35			
	AB	0.07			
	O	0			

mosquito repellents, and mosquito nets did not demonstrate a significant correlation. Our study report contrasting results with the study conducted in Ethiopia [27]. The use of anti-malaria spray in homes has been identified as an effective method for decreasing the risk of malaria. The health of individuals can have a significant impact on the probability of individuals to suffer from malaria as well. Winskill *et al.* [28] conducted research study in north-east Tanzania and using anti-mosquito nets demonstrated to be highly effective in preventing malaria, reducing the risk by 25%, children aged 5 to 13 exhibited a 71% increased risk of malaria compared to those under five, because they were less likely to sleep under insecticide-treated mosquito nets compared to younger children. Similarly, another study was conducted in Charsadda and Swabi districts of Pakistan reported contrasting results which demonstrated that the effective implementation of barriers such as window screens,

housing types, and bed nets significantly decreases the incidence of malaria [29]. The study documented episodic fever in 8.1% of patients, followed by chills in 3.1% and other related symptoms in 3.96% of the patients. Laboratory findings do not report anemia in any of the participating patient.

4. CONCLUSIONS

The current study reported that patients having blood group B are more vulnerable to *Plasmodium* infection. Participants sleeping in open environments were commonly affected due to exposure to vector-related factors, and those who sleep indoors but are affected by Malaria are due to lack of protective measures, such as using mosquito nets and mosquito repellent coils. This study shows the prevalence of malaria in a number of cases but is not statistically significant in the District Battagram. The findings of this study will be helpful in raising awareness among the local population about the association between blood group, sleeping area, and the risk factors associated with malaria.

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6. ETHICAL STATEMENT

The above study was conducted in accordance with the ethical standards of the institutional and national research committee. Before the data collection, ethical approval (106A) was obtained from the Department of Zoology, Government Post Graduate College, Mandian Abbottabad, Pakistan. The objectives of the research were clearly explained to each participant, and their privacy was ensured throughout the research process. Participation was voluntary, and participants had the right to withdraw at any time. No personal identifiers were used in the data analysis or reporting, and all data collected were stored securely and used solely for academic purposes only.

7. CONFLICT OF INTEREST

There is no conflict of interest regarding the publication of this paper.

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