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Original Article

Identification of Dengue Virus through Sero-Epidemiological Methods in Individuals of District Tank, Khyber Pakhtunkhwa

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Abstract

This study aimed to evaluate the serological characteristics of patients infected with dengue in 2024 at District Tank, Khyber Pakhtunkhwa, Pakistan. In 2024, a total of 100 individuals visited the hospital. Blood specimens from patients in the pathology laboratory. The data on dengue infection was obtained from the District Health Office in Tank. In the designated dengue patient ward, a patient with dengue fever who was showing little symptoms was admitted. For a period of five months, beginning in August and ending in December, the data was collected from hospitals. It was necessary to utilize a distinct form to consider the gender and history of the users. Furthermore, the Immunochromatographic Technique (ICT) was used in the laboratory screening of a patient suspected of having dengue fever. The collected blood samples underwent screening for Dengue infection utilizing the BIOLINE Dengue NS 1 Ag+Ab Combo Test. A total of 100 blood samples underwent screening. Out of the total samples, 28 tested positives for dengue fever, representing a percentage of 28%, while 72 samples, accounting for 72%, were found to be negative. Regarding patient age groups, 22 patients were found positive for Dengue infection, aged between 15 and 35 years, representing 78.57%, which constitutes most Dengue-infected patients. A significant epidemic of Dengue severity among patients was seen in September and October, attributed to the wet and post-rainy season. It is determined that biological, environmental, pharmacological, and educational strategies should be employed to combat illness. In addition, there is a requirement for the formation of teams to investigate the true origin of dengue disease and its potential effects on the residents of the region.

Keywords: Dengue Virus, Sero-Epidemiological Methods, NS1, IgG, IgM, Outbreak 2024.

Introduction

The Spanish term "dengue" implies "fastidious." It was first mentioned in 1789 and comes from the term "dinga," which implies an ill of spirit. The dengue virus, an enveloped positive single-stranded RNA virus that is a member of the family and genus Flavivirus, is the cause of dengue fever (Ul-Rahman *et al.,* 2024). This group also encompasses the hepatitis C virus, West Nile virus, and yellow fever virus. These viruses are highly dangerous and can inflict significant harm on human health (Kanwal *et al.,* 2023). Dengue is a leading cause of hospitalization, with an estimated 500,000 individuals infected with severe dengue requiring medical attention, predominantly affecting children. The annual mortality rate stands at 2.5% (Hushmandi *et al.,* 2024). The Aedes genus of mosquitoes is recognized for transmitting dengue disease, with the three predominant species being Aedes aegypti, Aedes albopictus, and Aedes Polynesians. A. aegypti, the primary vector, predominantly breeds in containers and has diurnal biting behaviour, inhabiting tropical and subtropical regions (Fernández-Salas *et al.*, 2015).

They primarily repose indoors in living rooms and bedrooms, exhibiting activity throughout twilight and daybreak. This enhances human-vector interaction and complicates the management of disease transmission (Bowman, Donegan, & McCall, 2016). A. albopictus and A. Polynesiansis act as vectors depending on their geographic distribution (Dávalos-Becerril *et al*., 2019). Africa, the Middle East, Europe, and the Americas are now home to A. albopictus, which was once only found in Southeast Asia, the Western Pacific islands, and the Indian Ocean. This increases the likelihood of epidemics of arboviral diseases in these countries. The eggs of Aedes mosquitoes demonstrate impressive durability, enabling them to survive prolonged phases of dryness. The inadequate management of waste and the lack of effective wastewater drainage systems lead to increased mosquito populations. Significant increases in mosquito larval populations are recorded during and after the rainy season. This results in post-monsoon dengue outbreaks in various countries throughout South and Southeast Asia (Ferreira-de-Lima & Lima-Camara, 2018). Increased temperature and humidity diminish the duration of virus replication in female mosquitoes. When an infected human bites an adult female mosquito, dengue viruses enter the insect and get access to multiple tissues. Viruses found in infected mosquitos' salivary glands are transmitted to humans during the following blood meal. Infected mosquitoes require a longer blood meal, increasing A. aegypti's efficiency as a dengue virus vector. There is evidence of both male-to-female and transovarial sexual transmission. This enables the virus to pass on its genes to subsequent generations even when no vertebral hosts are present, such as during an interepidemic (Frasca *et al.,* 2024).

Dengue fever is severe and affects children, babies, and adults, yet it has a low fatality rate. Dengue symptoms normally appear after 10-14 days of incubation following a mosquito bite and can continue for up to 2-7 days. If a high fever of greater than 40°C/104°F continues and is accompanied by chills during the febrile phase, which typically start on day 4 to 7 and stay for three to seven days, dengue should be diagnosed (Piedrahita *et al*., 2018). Fever is defined by a sudden onset and high-grade temperature exceeding 38.5°C, accompanied by migraine, retro-orbital pain, myalgias, and arthralgias. Fevers, skin rashes, nausea, and vomiting (along with other signs and symptoms). Fifty percent of patients experience lymphadenopathy, hepatomegaly, and maculopapular rash; the latter is more common during the early stages of Dengue infection and usually appears two to five days after the fever starts. Respiratory symptoms can manifest as a cough, nasal congestion, and a sore throat. Furthermore, leukopenia, thrombocytopenia, and transaminitis may be observed (Mohsina *et al*., 2022).

Significance of Study

The dengue virus (DENV) infection presents a considerable public health concern in tropical and subtropical regions, including Pakistan. District Tank, situated in Khyber Pakhtunkhwa, presents a scarcity of accessible data concerning the actual prevalence of dengue infections. This study offers important sero-epidemiological insights regarding the prevalence of dengue virus exposure among individuals in this region. By identifying the patterns of seropositivity across various age groups and demographics, the findings can assist local health authorities in crafting targeted prevention strategies, enhancing resource allocation, and fortifying disease surveillance systems. Moreover, the prompt identification of at-risk groups might facilitate the creation of more efficacious community awareness

initiatives and vector control programs, therefore diminishing the occurrence and effects of dengue outbreaks in District Tank and other susceptible regions.

Literature Review

In rural areas of Southeast Asian and Pacific Island dengue-endemic countries, A. albopictus serves as a dengue maintenance vector. Except in places like parts of China, the Seychelles, Japan, and Hawaii where A. aegypti is not present, A. albopictus is not an urban vector of dengue (Roy & Bhattacharjee, 2021).

In between epidemics, condensation has caused more severe dengue cases in Pakistan (Riaz *et al.,* 2024). The National Institute of Health (NIH) Islamabad has documented the incidence of cases in Pakistan for the years 2017, 2018, 2019, and 2020, recording figures of 22,938, over 3,200, 24,547, and 3,442, respectively. In 2020, just 1,153 occurrences were documented within the same timeframe. As of the conclusion of November 2021, a total of 48,906 cases have been registered across the nation. As of the conclusion of November 2022, a total of 75,450 cases had been documented in Pakistan, with the highest incidence occurring in KPK. From November 2021 to December 2021, a total of 16,388 cases were reported (Daniyal, Ogundokun, Abid, Khan, & Ogundokun, 2020).

Dengue fever affects around 3.9 billion people annually and is common in 128 countries, mostly in poor nations such as Pakistan. This research identifies DENV-2 as the primary serotype that has been both pandemic and dominant in Pakistan from 1985 to 1994. Like previous viral epidemics, the developing coronavirus pandemic also reduced dengue infections in 2020. The rapid and substantial rise of dengue in Pakistan is attributable to several factors, including overcrowding, rapid urbanization, travel, Insufficient vector control in endemic regions, along with inadequate healthcare and management systems. Additional research and investigation are necessary to create antiviral treatments or vaccines that confer serotype-specific protective immunity.

Method

The district is located between the latitudes of 31 degrees 15 minutes and 30 degrees 31 minutes North, as well as the longitudes of 70 degrees 22 minutes East. Located to the northwest of the Indus River and in proximity to the Takht-i-Sulaiman Mountain range, this area is characterized by its formidable and treacherous upland terrain, which has thwarted the ambitions of numerous kings and renowned warriors alike. There is a semiarid climate with scorching summers (with a maximum of 420 degrees Celsius in June) and chilly winters (with a low of 4.0 degrees Celsius). Tank experiences an average yearly temperature of 24.3 °C. There is an average of 260 mm of rain per year. Between May and June, humidity levels are at their lowest. Hot winds and dust storms are common throughout these months. In 1878, the Tank was elevated to the rank of Tehsil within the Dera Ismail Khan district. In 1992, it was elevated to the rank of district while still being part of the Dera Ismail Khan division.

Gathering of Clinical Information

The data on dengue infection was obtained from the District Health Office in Tank. In the designated dengue patient ward, a patient with dengue fever who was showing little symptoms was admitted. Every dengue patient was evaluated in detail for hepatomegaly, vomiting, diarrhea, epistaxis, and abdominal pain. Anti-dengue antibodies NS1, IgG, and IgM were among the diagnostic tests used in the study to assess liver function, platelet count, and dengue detection.

Laboratory Examination

The Immunochromatographic Technique (ICT) was used in the laboratory screening of a patient suspected of having dengue fever. The screening employed recombinant NS1 antigen in conjunction with coated IgG and IgM antibodies. These events took place between August and December of 2024.

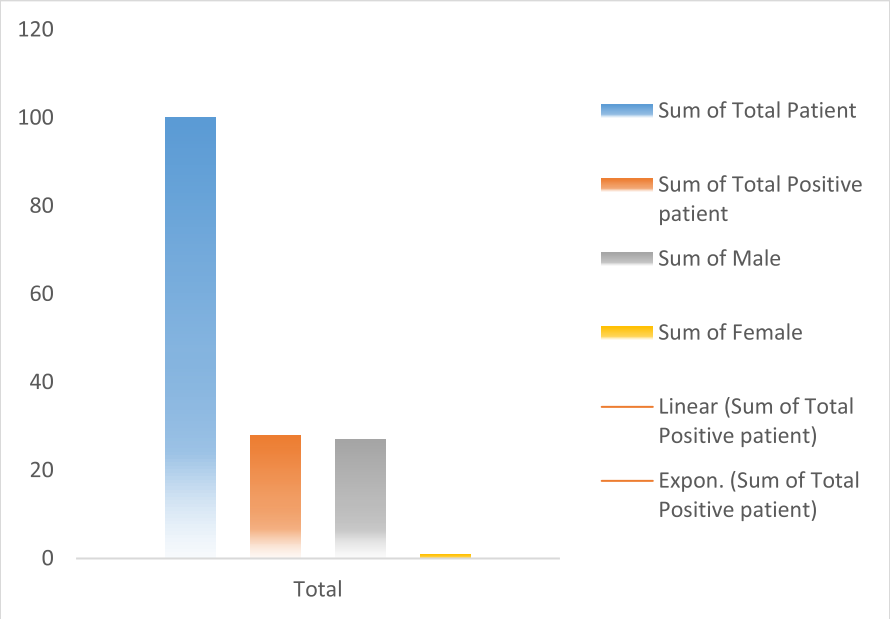
Results and Findings

A total of 100 suspected patients visited MMM Teaching Hospital Dera Ismail Khan for Dengue hemorrhagic fever from District Tank, presenting diverse symptoms, between August 2024 and December 2024. The pathology section recorded 28 positive cases of dengue. The highest frequency was observed in males at 27 (96.42%) compared to females at 1 (3.57%), as illustrated in Table 1.

Table 1
Prevalence of Dengue Infection

Total Patient	Total Positive Patient	Male	Female
100	28	27(96.42%)	01(3.57%)

Figure 1
Prevalence of Dengue Infection



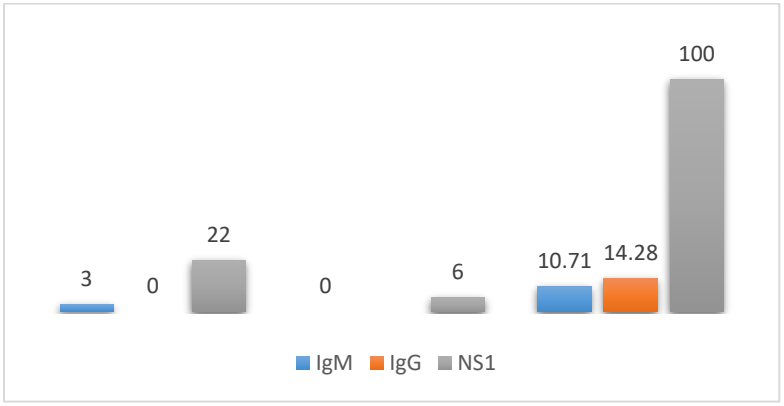
Distribution of Dengue Infection by Age

Patients infected with dengue were categorized into three groups: ages 0-15, 16-35, and 36-60. The age group 16 to 35 exhibited the highest incidence of dengue fever, with 46 patients accounting for 48.42%. This was followed by the 1 to 15 age group, which had 20 patients (21%), the 36-50 age group with 16 patients (16.84%), and those aged 50 and above, totaling 13 patients (13.68%). Age-wise distribution is detailed in Table 2.

Table 2
Dengue fever incidence by age group Positive individuals

Anti-Dengue Antibodies	0-15 Years	16-35 Years	36-60 Years	Percentage %
IgM	0	03	0	10.71%
IgG	0	0		14.28%
NS1	0	22	06	100%

Figure 2
Age-wise Prevalence of Dengue Positive Individuals



Antibodies based Distribution of Dengue Infection

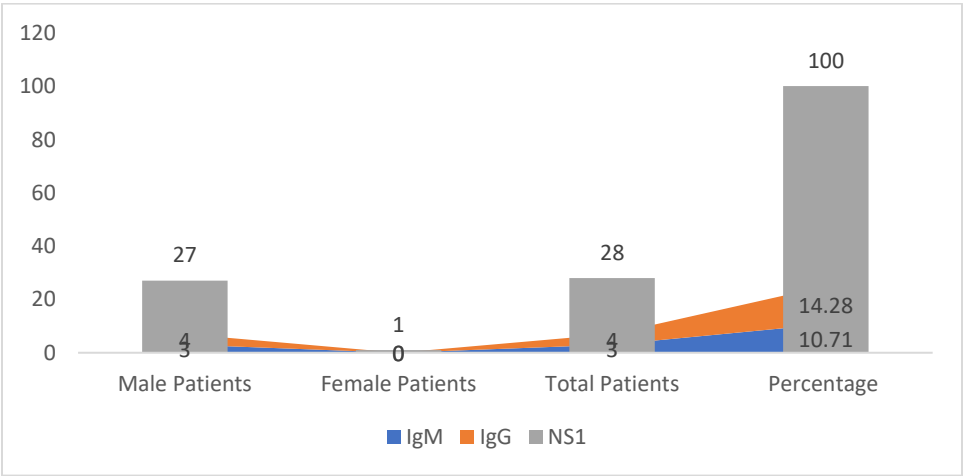
The serological test markers for all 28 positive patients are presented, including NS1, IgG, and IgM, as detailed in Table 3.

Table 3

Prevalence of dengue fever in relation to gender and anti-dengue antibodies

Anti-Dengue Antibodies	Male Patients	Female Patients	Total Patients	Percentage
IgM	03	NA	03	10.71%
IgG	04	NA	04	14.28%
NS1	27	01	28	100%

Figure 3
Prevalence of dengue-positive individuals by gender and anti-dengue antibodies



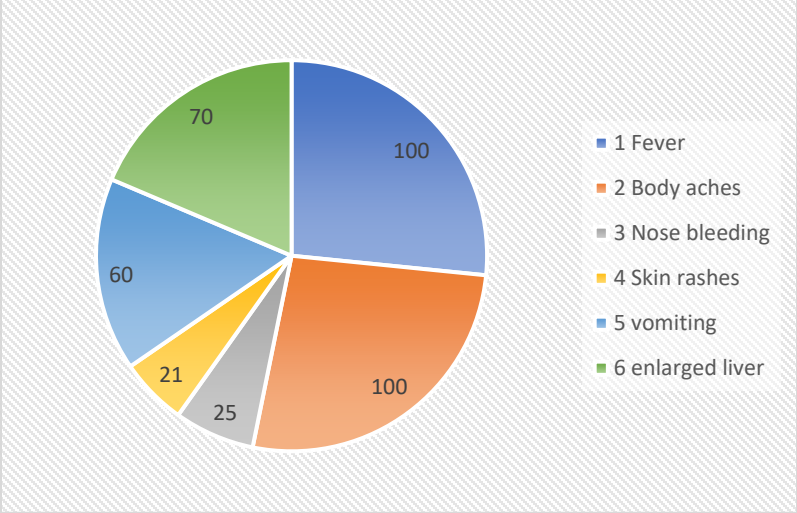
Clinical Features of Dengue Fever in Patients

The dengue fever patient exhibited various signs and symptoms, including fever, nasal bleeding, vomiting, hepatomegaly, and skin rashes. Refer to Table 4.

Table 4
Clinical features and symptoms of Dengue infected Patients

S. No	Signs & Symptoms	Percentage
1	Fever	100%
2	Body aches	100%
3	Nose bleeding	25%
4	Skin rashes	21%
5	vomiting	60%
6	enlarged liver	70%

Figure 4
Clinical features and symptoms of patients infected with Dengue



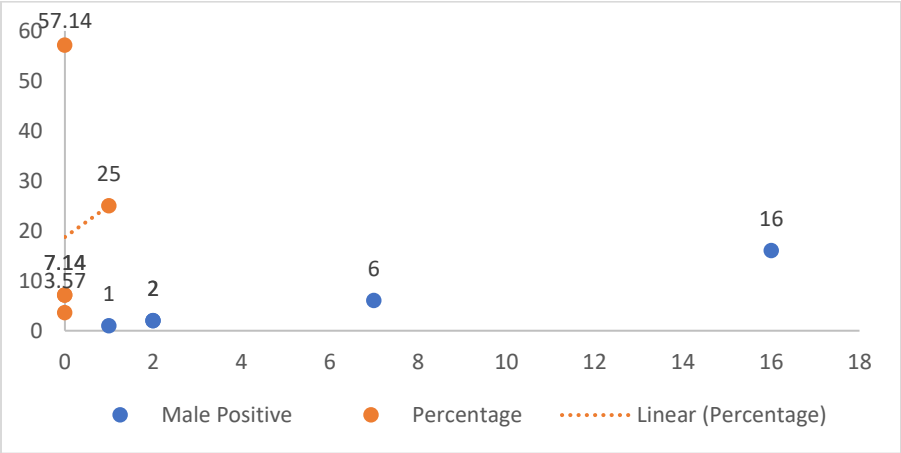
Month Wise Distribution of Dengue Infection

Dengue outbreaks are also reported from August to December 2024. The spreading rate was particularly high in October and December (57.14% and 25%, respectively), followed by September and November (7.14% in both). The spread rate that was reported in August was the lowest, at 3.57%, as illustrated in Table 5

Table 5
Month-wise Dengue Fever Distribution

Months	Total Positive Cases	Male Positive	Female Positive	Percentage
August	01	01	0	3.57%
September	02	02	0	7.14%
October	16	16	0	57.14%
November	02	02	0	7.14%
December	07	06	01	25%

Figure 5
Month Wise Distribution of Dengue Fever



Discussion

The dengue virus may have entered Pakistan via tires with mosquito eggs from Karachi ports. Dengue has caused multiple epidemics in Pakistan. There were 12 confirmed incidences of dengue fever out of 174 patients in Punjab, Pakistan in 1982. There were no reliable records of dengue infections in Pakistan prior to 1994. Abdominal pain was the most frequently observed symptom during the infection, reported in 51% of the patients. Additionally, 94% of the individuals were reported to have splenomegaly. Many of these patients were anemic almost certainly. Their Hb level was less than 10g% and their platelets fell between 50,000 and 100,000cmm. Such findings have also come across in the present study (Rehman, Haq, Asghar, Afridi, & Faisal, 2020). The initial outbreak of dengue virus infection in Pakistan was recorded in 1982, specifically in the Lahore district of Punjab province. World Health Organization Since 2013, numerous outbreaks have been identified in the provinces of Khyber Pakhtunkhwa, Sindh, and Baluchistan in Pakistan. Nevertheless, there has been a lack of focus on developing a comprehensive laboratory-based surveillance program to understand the impact of circulating viral serotypes. This understanding could significantly enhance the primary efforts in disease monitoring and control. Due to inadequate consideration, two significant DENV outbreaks occurred in 2011 and 2013 across various districts in Pakistan, specifically in Khyber Pakhtunkhwa's Swat and Mansehra districts, as well as in Lahore and its surrounding areas in Punjab. Our study revealed a higher prevalence rate of 14.50% (69 positive cases) among males compared to 5.46% (26 positive cases) among females, as illustrated in Table 1. In this study, positive dengue cases were classified into four age-based groups. The highest prevalence is 16–35, with 46 instances (48.42%). This is followed by the 1 to 15 age group, which has 20 cases (21%). The 36 to 40 age group shows 16 cases (16.84%), while the above 50 age group has the least number of cases, totaling 13 (13.68%) (Khan *et al.*, 2021). In this research, we identified antibodies and antiantibodies that were like NS1, IgG, and IgM in various laboratory analysis instruments. The highest ratio of NS1 is (100%), followed by IgG (14.28%). A research initiative undertaken in Khyber Pakhtunkhwa revealed that a total of 612 individuals were identified as suspected cases. Dengue antibodies, both IgG and IgM, were 52.12% prevalent, with 23 samples (3.75%) having both. Patients visiting endemic areas is the main cause.

Conclusion

Dengue fever is an endemic disease in Pakistan, occurring year-round. The biggest number of occurrences was found to take place during the months of October, September, and November, respectively, according to the findings of the investigation. The correlation between climate change and vector-borne diseases in Pakistan necessitates examination, as these elements substantially influence the timing and severity of dengue and other disease outbreaks. To properly diagnose and treat the illness, more research on hematological markers is needed. Implementing awareness efforts

centered on vector control and sewage management, as well as taking societal issues into account for better disease control and eradication, is strongly recommended to manage dengue outbreaks.

Recommendations

1. **Establish Sentinel Surveillance Sites:** Implement sentinel surveillance locations in District Tank to continuously monitor dengue activity and facilitate early epidemic detection.
2. **Enhance Diagnostic Facilities:** To guarantee prompt and precise diagnosis, provide local healthcare facilities with rapid diagnostic tests (RDTs) for dengue NS1 antigen, IgM, and IgG antibodies.
3. **Community Engagement:** Initiate community-oriented activities to eradicate mosquito breeding habitats, including stagnant water in containers, tires, and drainage systems.

By adopting these recommendations, District Tank and analogous areas can enhance their ability to combat dengue, diminish illness burden, and elevate public health outcomes. The results of this study can provide a basis for evidence-based strategies and policies to combat dengue in Khyber Pakhtunkhwa and elsewhere.

Limitations and Future Research Directions

The study only gives an overview of dengue seroprevalence at a particular moment in time, which limits understanding of seasonal patterns or long-term transmission dynamics. Longitudinal data would better reflect swings in dengue incidence. The study's focused on District Tank limits its applicability to other areas of Khyber Pakhtunkhwa or Pakistan. Furthermore, data gathered during non-outbreak times may underestimate genuine prevalence. Further researchers can conduct study with larger sample size from different regions and both rural and urban to get better understanding and to generalize the results on larger population.

Declarations

Ethical Approval and Consent to Participate: This study strictly adhered to the Declaration of Helsinki and relevant national and institutional ethical guidelines. Informed consent was not required, as secondary data available on websites was obtained for analysis. All procedures performed in this study were by the ethical standards of the Helsinki Declaration.

Consent for Publication: Not Applicable

Availability of Data and Material: Data for this study will be made available upon a request from the corresponding author.

Competing Interest: The authors report no competing and conflict of interest.

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