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Risk Factors, Screening and Seroprevalence of Dengue Virus Antigen (NS1) in Clinically Suspected Patients: A Community-Based Hospital Study

Shah Zeb*[1], Saddam Hussain[2], Nuzhat ul Ain*[3], Muhammad Wajahat[4], Jawad Ullah[5], Ahsan Naeem[6], Kiramat Ullah[7], Bibi Salma Tassaduq Naqvi[8]

[1]Faculty of Biomedical & Health Sciences, Department of Microbiology, University of Haripur

[1]Department of Microbiology, Pakistan Kidney & Liver Institute and Research Center, Lahore

[2]ST2 on IMG Scholarship Program, General Surgery, Royal College of Surgeon Ireland

[3]Department of Microbiology, Asian Medical Institute, 58 Gagrina Street, Kant, Kyrgyzstan, Russia

[4]Faculty of Biomedical & Health Sciences, Department of Microbiology, University of Haripur

[5]Department of Microbiology, Hazara University, Mansehra

[6]Faculty of Biomedical & Health Sciences, Department of Microbiology, University of Haripur

[7]Department of Microbiology, Hazara University, Mansehr

[8]Faculty of Biomedical & Health Sciences, Department of Microbiology, University of Haripur

Corresponding authors

Shah Zeb

ORCID: https://orcid.org/0000-0002-5156-9243

Nuzhat ul Ain

nuzhataslam.3105@gmail.com

Abstract

Objectives: The study aims to find out the highly exposed age group, gender, area risk factors and serological marker.

Study Design: Cross sectional

Study Area: The Study was conducted at Civil Hospital from July 2022 to September 2022.

Methodology: All the tests were performed by the AccessBio® kit methodology.

Results: About 945 positive patients were included in the study for diagnosis, of whom 594 were Male and 351 were Female. Fever calculated with p= 0.007, a rapid, weak pulse, pallor or cool skin, rashes calculated like p= 0.005, headache with p= 0.001. We reported following groups 18-30yrs (718, 75.9%), 30-40yrs (103, 10.8%), 40-50yrs (45, 4.76%), and 50-60yrs (79, 8.3%). M=236, SD=278, Variance, σ_2= 77785.6 and CI=95%, $1.960\sigma\bar{x}$ 236.25 ±273.323(±115.69%). The affected areas such as (Barradaar 41, 4.3%), (Chouiyan 76, 8.04%), (New Darband 423, 44.7%), (Naryaani 27, 2.87%), (Kajla 52, 5.50%), (Garh-Bala 118, 12.4%), and (Bandi 115, 12.1). M=118.1, SD=119.3, Variance, σ_2= 14248.6, CI=95%, $1.960\sigma\bar{x}$ 118.125 ±82.717 (±70.03%), and probability at 0.004. In males, IgG/IgM (27, 4.5%), IgM/NS1 (314, 53.8%), IgG/NS1 (193, 32.4%), and IgM with 60, 10.1%). M=148.5, SD=113.9, Variance, σ_2=

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12991.25, CI=95%, 1.960σx̄ 148.5 ±111.7 (±75.22%), and probability was 0.003. In female IgG/IgM (61,017.3%), IgM/NS1 (37, 10.5%), IgG/NS1 (75, 21.3%), and IgM with 178, 50.7%). M=87.7, SD=53.8, Variance, σ2= 2899.68, CI=95%, 1.960σx̄ 87.75 ±52.772 (±60.14%), and probability was 0.003.

Conclusion: We screened the most affected area such as New Darband, a higher seroprevalence of NS1 was found in males as compared to females and the highly exposed age group was 18-30yrs.

Keywords: Dengue endemic, Seroprevalence, NS1, New Darband, Panic fever

Background

The dengue virus belongs family Flaviviridae within the genus Flavivirus. Flavivirus comprises the most significant gathering of arthropod-transmitted infections, including significant human viruses like dengue(1), (2). Dengue virus contains positive-sense single-stranded RNA. The length of RNA is 11kb and enclosed by enveloped. This virus consists of a single reading frame that is responsible for the synthesis of three structural and seven non-structural proteins (3). It is characterized by high fever, muscle pain, severe headache, nausea vomiting, and rash appearing on the skin. In the case of dengue bone break fever is a common symptom (4). In severe cases, bleeding comes from the nose, and mouth due to a blood vessel burst. Platelet cell number decreases due to this infection. In the case of dengue shock syndrome frequent vomiting, heavy bleeding, and sudden blood pressure decrease. The symptoms of dengue hemorrhagic fever and dengue shock syndrome lead to death (5).

Dengue virus Critical disease is characterized as the underlying or first infection with a firm serotype. The majority of critical illnesses are generally asymptomatic or show as a gentle febrile sickness, although they can likewise cause hemorrhagic fever in certain patients, particularly in newborn children brought into the world by Dengue virus. Ensuing disease with an alternate serotype is known as optional dengue infection and may prompt serious clinical suggestions, for example, dengue hemorrhagic fever (DHF) or dengue shock disorder (DSS) (7).

Every year more than 50 million people are affected by dengue hemorrhagic fever. This disease is a bad effect on the economy (8). The incubation period of this virus is 3 to 7 days. Symptoms are divided into three phases. An initial phase of infection in which includes high fever, malaise, loss of appetite, vomiting, and chills. It is also known as the febrile phase. The second phase is a critical phase in which the virus spreads systematically and two more severe symptoms are dengue hemorrhagic fever, and dengue shock syndrome which leads to death, and the third phase is the recovery phase (9). Two theories are circulating about dengue hemorrhagic fever and dengue shock syndrome. According to one theory, this syndrome is caused by more virulence strains of the dengue virus. While in another theory this is a normal strain of the dengue virus but the due abnormal immune response of the host leads to this syndrome. In dengue infection firstly patient

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suffers from fever for 2-7 days. When body temperature becomes normal level but the patient is still ill at this stage shock occurs. Suddenly platelets amount reduces below 100,000/mm³. This is known as dengue hemorrhagic fever (10). In the case of dengue shock syndrome increased vascular permeability as a result leakage occurs in a blood vessel and blood comes from the patient nose. Space fluid released and loses electrolytes such as potassium, and sodium ions and leading to sudden blood pressure. Due to viremia complication occurs in multi-organ such as pleural effusions, pericardial effusions and hypotension that lead to death (11).

Materials and Method

Study area

New Darband Township has located nearby the Indus River, which lies under tropical areas of the Mansehra with 38-42 ° c temperatures. New Darband counts in a rural area and is famous for ponds, rivers, and tube wells. This study was conducted in the Laboratory of Civil Hospital, New Darband Township

Selection of patients

We isolated around 945 patients for the research project who were profoundly suspected and were truly Dengue fever. Every one of them was arranged into several groups depending on age, sex, and area. Blood tests had been drawn from infected patients with dengue-like sicknesses and suspected patients as well. The specimen was dissected by following the Standard protocols for determination, every individual specimen was tried for the occurrence of dengue virus IgM/IgG antibodies, and NS1 antigen.

Specimen collection and processing

Fresh blood was obtained about 2ml in volume from patients by puncturing the vein. Blood stored in purple EDTA tubes (K3), 20ul whole blood was added to AccessBio® rapid diagnostic kit to each well for the detection of IgG/IgM and NS1 antigen.

Ethical approvals

The Ethical approval enlistment no MIC-UOH002 was delivered by the Ethical committee leading group of a civil hospital, New Darband as well as from the Department of Microbiology, the University of Haripur under registration no F20-2013. The concerned office permits us to direct the clinical examination and testation under the act and regulations of ethics, additionally, they supervised all the material that we utilized in the investigation of the diagnosis. They permit us in the wake of depicting the cause and aim of the study. They formally handed off an ethical letter to us to proceed with the research work precisely.

Consent Consideration

We included in a study about 945 questionnaires as per sample size or study population. Each Questionnaire was carrying fewer questions according to the Demographic, Health, Previous

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history, Occupation, and dengue fever. We generated the questionnaire in an understandable language called English as well as in a local language who are unable to understand it. As all the patients did to answered, hence at the end they also release their signatures as well as cell number.

Statistical significance

The isolated Data such as questionnaire data (Age, sex, Areas, Medical history, routine Questions, and dengue-related questions were interpreted through Microsoft Excel by applying specific formulas such as Mean and standard deviation, variance, and probability for Different groups of patients data based on age, gender as well as Dengue serological markers like IgG/IgM and NS1.

Methods in Study
Serological Detection

Blood samples of 945 patients were tested in the Diagnostic center, civil hospital New Darband for the etiology of dengue. All the tests were performed by the AccessBio® kit methodology. One of AccessBio®'s great missions is to improve the lives and well-being of people around the globe. Through the spreading out of in vitro diagnostic tools and technology, they have successfully commercialized high-quality products to fight malaria, dengue, Corona, and other serious diseases.

Antibodies test: IgM/IgG and NS1 rapid test kit

We used AccessBio® a rapid vital diagnostic kit for the detection of IgG and IgM and NS1 Antigen tests for the dengue virus. We used these tests initially for the detection of current or previous infections. The device detected different classes of antibodies (IgM, IgG), which are produced by the body against dengue fever infection. Perform a test after 5-8 days after exposure for effective detection of IgM antibodies. If IgM detection occurs, it means an infection is in the initial stage. The level of blood will increase for a few weeks, then slowly decrease IgM antibodies after months to detectable levels, next IgG antibodies will be produced slowly in response to infection and it is an indication of past infection.

NS1 Antigen test:

We used the AccessBio® NS1 kit which is commercially available as a diagnostic test kit, to detect the non-structural proteins NS1 for the presence of dengue viruses. During dengue infection secretion of NS1 is found in a portion of the body serum. In this test, synthetically labelled antibodies are used for the detection of the NS1 protein portion of dengue. NS1 and IgM both communally provide diagnostic results during 1-7 days of infection.

Results
The study area (Civil Hospital)

The Rural Health Center (Civil hospital), New Darband, served as the study's site. The only healthcare facility serving a large number of densely populated towns and villages (more than 20).

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Since the questionnaire was completed and we moved through with further diagnostic testing to find the particular antigen and antibodies against DEN-Virus by AccessBio®. About 945 positive patients were included in the study for diagnosis, of whom 594 were Male and 351 were Female.
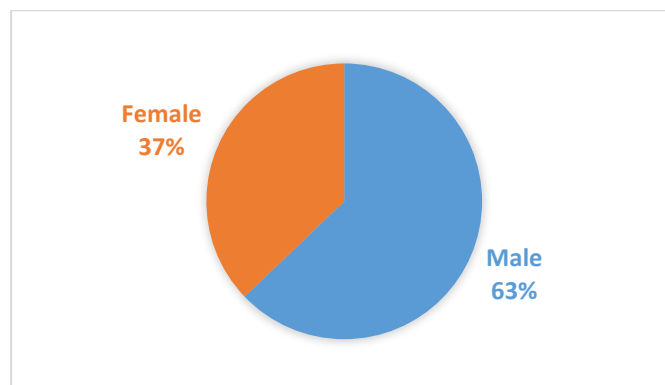

Figure no.1 Genders participated in the study

Clinical Indication of dengue

The study omitted the nature of the problems while including all positive patients. Only patients who had all of the warning signs, such as full-blown symptoms like fever calculated like p = 0.007, a rapid, weak pulse, pallor or cool skin, rashes calculated like p = 0.005, headache calculated like p = 0.001, joint pain, leukopenia, abdominal pain, retro-orbital pain, persistent vomiting, vomit that contains blood, anorexia, and postural hypotension, were included in this study.
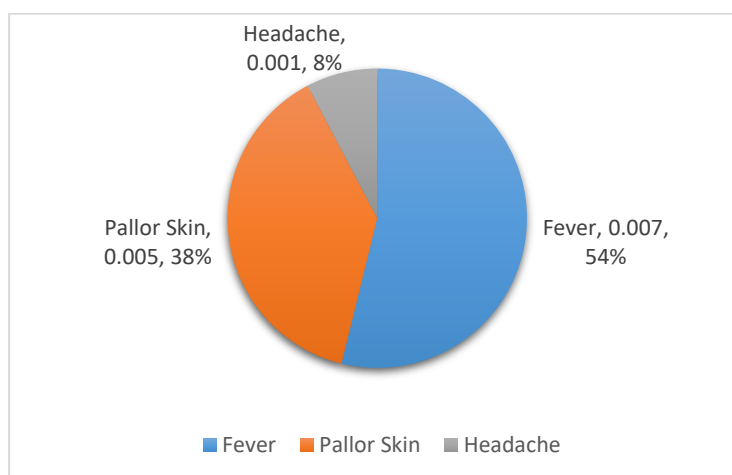

Figure no.2 Clinical signs & symptoms reported in suspected patients

Age group

We reported following groups 18-30yrs (718, 75.9%), 30-40yrs (103, 10.8%), 40-50yrs (45, 4.76%), and 50-60yrs (79, 8.3%). The Mean was calculated against age groups such as M=236, SD=278, Variance, σ^2= 77785.6 and Cl=95%, 1.960$\sigma\bar{x}$ 236.25 ±273.323(±115.69%).

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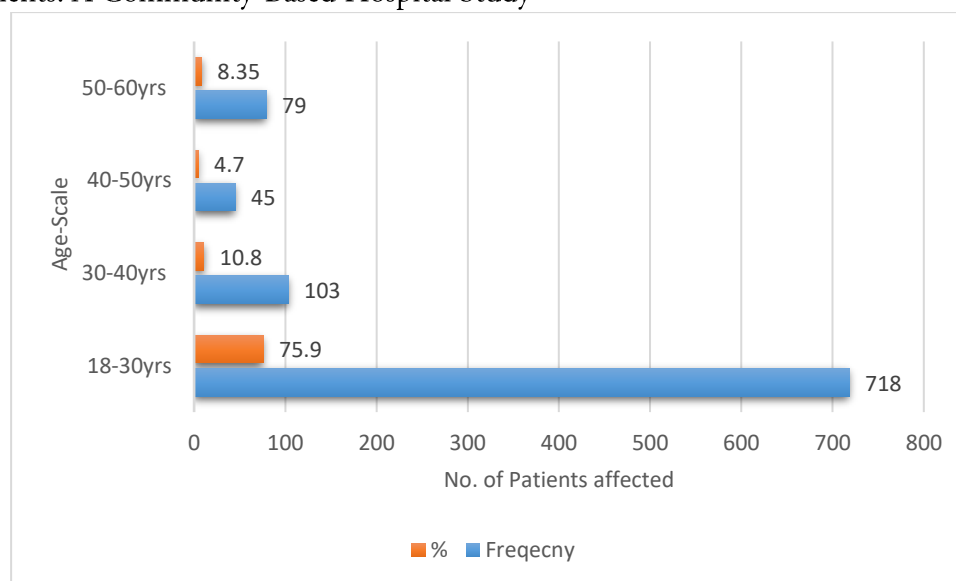Suspected Patients: A Community-Based Hospital Study

Figure no.1 Age-groups of study patients

Area-wise distribution

we added different areas under the circle of the civil hospital, such as (Barradaar 41, 4.3%), (Chouiyan 76, 8.04%), (New Darband 423, 44.7%), (Naryaani 27, 2.87%), (Kajla 52, 5.50%), (Garh-Bala 118, 12.4%), and (Bandi 115, 12.1). The Mean was calculated for areas such as M=118.1, SD=119.3, Variance, $\sigma 2$= 14248.6, Cl=95%, $1.960\sigma\bar{x}$ 118.125 ±82.717 (±70.03%), and probability at 0.004.

Table no.1 Affected area and its distribution of positive dengue cases

Area	M	F	sum	%	M±SD	Variance	P-value
Barradaar	30	11	41	4.338624			
Chouiyan	55	21	76	8.042328			
New Darband	214	209	423	44.7619			
Kajla	44	8	52	5.502646	118.1(119.3)	14248.6	0.004
Naryaani	18	9	27	2.857143			
Garh Bala	80	38	118	12.48677			
Dokan	71	22	93	9.84127			
Bandi	82	33	115	12.16931			

Detection of dengue viral markers by AccessBio®

All the serological markers (IgG, IgM and NS1) were detected by the AccessBio® (United States of America) due to its highest specificity and sensitivity rates (figure no.1). In this study, we focused on the only patients who were tested positive for the dengue viral fever or dengue viral markers (IgG, IgM and NS1).

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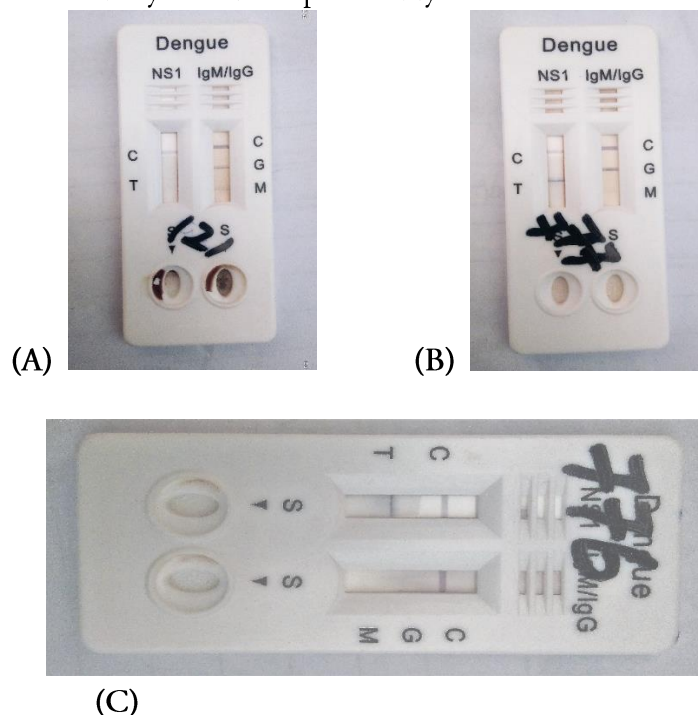Suspected Patients: A Community-Based Hospital Study

Figure no.1 Demonstration of *AccessBio*® devices (a) tested for IgG and IgM positive, while
(b) IgG and NS1 Positive (c) *AccessBio*® NS1 Positive device

We found a higher seroprevalence of dengue-like NS1 (Non-structural protein 1) across the tested
population for Den-V with (females 14.1% while males with 75%), instead of IgG and IgM
antibodies. In males, we screened dengue viral markers like IgG/IgM (27, 4.5%), IgM/NS1 (314,
53.8%), IgG/NS1 (193, 32.4%), and IgM with 60, 10.1%). The Mean was calculated for males
such as M=148.5, SD=113.9, Variance, $\sigma2$= 12991.25, Cl=95%, 1.960$\sigma\bar{x}$ 148.5 ±111.7
(±75.22%), and probability was 0.003. In females, we screened dengue viral markers like IgG/IgM
(61,017.3%), IgM/NS1 (37, 10.5%), IgG/NS1 (75, 21.3%), and IgM with 178, 50.7%). The
Mean was calculated for females such as M=87.7, SD=53.8, Variance, $\sigma2$= 2899.68, Cl=95%,
1.960$\sigma\bar{x}$ 87.75 ±52.772 (±60.14%), and probability was 0.003.

Table no.2 Detection and comparison of dengue viral markers in patients

Males	f	%	M+SD	Females	f	%	M+SD
IgG/IgM	27	4.545455		IgG/IgM	61	17.3789174	
IgM/NS1	314	52.86195		IgM/NS1	37	10.5413105	
IgG/NS1	193	32.49158	148.5(113.9)	IgG/NS1	75	21.3675214	87.7(53.8)
IgM	60	10.10101		IgM	178	50.7122507	

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Discussion

Dengue virus Critical disease is characterized as the underlying or first infection with a firm serotype. The majority of critical illnesses were generally asymptomatic or shown as a gentle febrile sickness, even though they can similarly cause hemorrhagic fever in certain patients, particularly in newborn children brought into the world to DENV-invulnerable moms. Ensuing disease with an alternate serotype is known as optional dengue infection and may prompt serious clinical suggestions, for example, dengue hemorrhagic fever (DHF) or dengue shock disorder (DSS).

We documented the endemic and high seroprevalence in New Darband with 423, 44.7%, due to high population burden, using their water from open ponds and rivers instead of filter and Sealed water, outdoor work, lack of awareness campaigns, lack of health resources and well-establish healthcare facilities. The Mean was calculated for areas such as M=118.1, SD=119.3, Variance, $\sigma2= 14248.6$, Cl=95%, $1.960\sigma\bar{x}$ 118.125 ±82.717 (±70.03%), and probability at 0.004.

In a cross-sectional study by Mahmood et al., about 274 healthy populations matured 15 years and above were arbitrarily chosen to utilize the multistage inspecting procedure. These people were met between July–September 2012, utilizing a semi-structured survey, trailed by drawing 3 mL of their venous blood for the dengue IgG test. Nova-Tech ELISA unit with affectability and particularity of (96.5% and 97.5%), individually, was utilized for serology (12).

Youngsters (18-30 years old, 718, 75.9) were the most infected or exposed group as a result of affected people working outdoors and a lack of knowledge about dengue transmission. We exposed the highly exposed age group that drew more attention by experiencing the dengue virus's blooming peak. The Mean was calculated against age groups such as M=236, SD=278, Variance, $\sigma2= 77785.6$ and Cl=95%, $1.960\sigma\bar{x}$ 236.25 ±273.323(±115.69%).

Ali et al. 2013 selected a sum of 612 subjects with a presumed infection. Out Of the 612 speculated cases (319 were discovered positive for dengue IgG, IgM, or both IgG and IgM). The overall weighted predominance of dengue viral antibodies IgM, as well as IgG, was (52.12%). Generally, of the (52.12%, 31.86% were positive for dengue IgM) and (20.26% were positive for dengue IgG) while Just 23 (3.75%) examples showed both (IgG and IgM antibodies). A higher predominance of IgM (39.35%) while, IgG (22.42%) antibodies were found in the age bunch (21–30 years) when contrasted with the youngster's age bunch (≤10 years) and the most established age bunch (≥51 years) (13).

According to Muhammad et al. Dengue seroprevalence was altogether recognized in male patient's n = 149, 87.13%, when contrasted with female patients' n = 22, 12.87%, P = 0.000 4. Patients in the age gathering of (20–39 years, n = 114, 66.67%), were more inclined to dengue, however less common in the age bunch over (60 years). Sex, age classification, and region were critical elements in the dengue seroprevalence P = 0.034 8. The general seroprevalence of anti-dengue (IgG was 56.60%, n = 171) in alluded dengue-suspected patients. Stale water repositories were found answerable for the spread of the infection preferring mosquito-rearing destinations (14).

From an aggregate of Ahmed, Ali, and Tabassum 900 subjects, 46% were found to have positive IgG in their blood. Their outcomes uncovered that a male offspring old enough over 10 years was

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bound to be IgG-positive. Other danger factors related to seropositivity included lower family pay and nonappearance of sickliness, thrombocytopenia, and absence of handwashing (15).

Seasonal variation of seropositive dengue patients from (2010 to 2013) by Zameer et al. showed the most prominent dengue fever disease onset found in October whereas last seen in the months from Jan to April. Age-wise younger subjects were more prone to the infection with a mean age of (21 to 30 years). 64% of the total febrile seropositive cases were male as compared to female patients noted at 36% (16).

Quadri et al. analyzed 378 cases among these 126 (33.3%) cases were positive for (anti-dengue IgG and IgM antibodies). Females were more affected, (266 70.4%) and the commonest age group affected in these adults was between (37-54 years), (143 37.8%). A significant association at (p-value<0.5) was found in (gender, ethnicity, marital status, primary education, and unemployed subjects with seropositive tests) (17).

Dengue prevalence was significantly detected by Mukhtar, and Iqbal et al in male patients (n = 149, 87.13% as compared to female n = 22, 12.87% (P = 0.000 4). Patients in the age group (20–39 years, n = 114, 66.67%) were more prone to dengue but less prevalent in the age group more than (60 years). Gender, age category, and area were significant factors in the dengue prevalence P = 0.0348. The overall prevalence of anti-dengue (IgG was 56.60% n = 171) in dengue-suspected patients. Stagnant water reservoirs were found responsible for the spread of the disease favouring mosquito breeding sites (18).

Conclusion

The study pointed out the endemic and seroprevalence under the circle of the civil hospital. The highest seroprevalence of dengue-NS1 was in males while it was lowest in females due to a lot of males working in tyer shops, garages, boat operators, and fishermen. They also slept outdoors due to the warm ecosystem around their living area. All of them utilized their water from open ponds and rivers instead of filtered or sealed water and worked outdoors. New Darband was highly affected due to its higher population rates, the Indus riverbank, and ponds that are found in large numbers.

Abbreviations

g/dl
ul/cumm
mg/ml milligram milliliter
DEN-V dengue virus
IgG/IgM immunoglobulin M and G
NS1 non-structural protein-1
Kg kilogram

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