



Comparative diagnostic analysis, frequency, severity, and seroprevalence of Dengue fever

Sameer Zafar¹, Samia Yousaf², Maria Irshad Chohan³, Shah Zeb^{4*}, Mohammed Garout⁵, Muhammad Zahid Ali⁶, Muhammad A. Halwani⁷, Mohammed Aljeldah⁸, Ahlam Alynbiawi⁹, Saleh A. Alshamrani¹⁰, Sarah A. Algosaibi¹¹, Roua A. Alsubki¹², Abdulmonem A. Alsaleh¹³, Ali A. Rabaan^{14,15,16,*}

¹ Department of Medical Education, Sharif Medical and Dental College, Lahore, Punjab, Pakistan

² Department of General Medicine, St Mary's Hospital, Isle of Wight NHS trust, UK

³ Department of Medical Education, Sheikh Zayed Medical College, Rahim Yar Khan, Punjab, Pakistan

⁴ Department of Microbiology, Pakistan Kidney and Liver Institute and Research Center, Lahore, Punjab, Pakistan

⁵ Department of Community Medicine and Health Care for Pilgrims, Faculty of Medicine, Umm Al-Qura University, Makkah 21955, Saudi Arabia

⁶ Department of Microbiology, Yahya Welfare Complex Hospital, Haripur.

⁷ Department of Medical Microbiology, Faculty of Medicine, Al Baha University. Al Baha 4781, Saudi Arabia

⁸ Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, University of Hafr Al Batin, Hafr Al Batin 39831, Saudi Arabia

⁹ Infectious Diseases Section, Medical Specialties Department, King Fahad Medical City, Riyadh 12231, Saudi Arabia

¹⁰ Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Najran University, Najran 61441, Saudi Arabia

¹¹ Academic and clinical Training, Eastern Health Cluster-Rural health network, Dammam 32247, Saudi Arabia

¹² Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud University, Riyadh 11362, Saudi Arabia

¹³ Department of Clinical Laboratory Sciences, Mohammed AlMana College of Health Sciences, Dammam 34222, Saudi Arabia

¹⁴ Molecular Diagnostic Laboratory, Johns Hopkins Aramco Healthcare, Dhahran 31311, Saudi Arabia

¹⁵ College of Medicine, Alfaisal University, Riyadh 11533, Saudi Arabia

¹⁶ Department of Public Health and Nutrition, The University of Haripur, Haripur 22610, Pakistan

***Corresponding author:** 1. Ali A. Rabaan, Molecular Diagnostic Laboratory, Johns Hopkins Aramco Healthcare, Dhahran 31311, Saudi Arabia, Email: namalik288@gmail.com 2. Shah Zeb, Department of Microbiology, Pakistan Kidney and Liver Institute and Research Center, Lahore, Punjab, Pakistan

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ABSTRACT

The study involved a total of 450 participants, with 359 males and 91 females. Out of a larger group of 4820 individuals, 4170 tested positive for dengue, while 650 tested negative. In the subsequent analysis, the negative cases were excluded, added only the positive cases. Signs and symptoms of dengue were analyzed, including fever ($p = 0.007$), rapid and weak pulse, pallor or cool skin, rashes ($p = 0.005$), and headache ($p = 0.001$). The mean difference between genders was 2085, with a standard deviation of 771 and a variance of 594441.

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The 95% confidence interval was $2,085 \pm 1,068.551$ ($\pm 51.25\%$), and the p-value was 0.002. The mean differences for specific markers related to dengue were as follows: IgM = 548.5, IgG = 294.5, NS1 = 949, and IgG/IgM = 293. The standard deviations for these markers were IgM = 71.5, IgG = 23.5, NS1 = 738, and IgG/IgM = 128. The 95% confidence intervals were calculated as $1.960\sigma_{\bar{x}}$, 521.25 ± 320.176 ($\pm 61.42\%$), with a variance of $V = 213479.75$ and a p-value of 0.009. The overall mean difference for dengue serotypes was 521.2, with a standard deviation of 350.4 and a variance of 122845.18. The 95% confidence interval was 521.25 ± 242.879 ($\pm 46.60\%$), with a p-value of 0.007. The mean \pm SD values for hemoglobin = 16.0 ± 2.41 , platelets = 71250.2 ± 24321.5 , and hematocrit = 47.3 ± 7.22 . In dengue hemorrhagic fever, the mean \pm SD values were hemoglobin = 17.2 ± 2.83 , platelets = 32412.5 ± 27530.2 , and hematocrit = 66.5 ± 9.72 . The study highlighted the endemic nature and seroprevalence of dengue within the vicinity of the civil hospital. The highest seroprevalence of dengue, indicated by the NS1 marker, was observed in males, while the lowest prevalence was seen in females, possibly due to more males working in tire shops and labor-intensive jobs.

Keywords: *Dengue Fever, Virus serotypes, diagnostics*

INTRODUCTION

The dengue virus is a Flaviviridae virus. Flavivirus includes important human viruses such as dengue (1)(2). Dengue virus has positive-sense RNA. 11kb of encapsulated RNA. This virus's single reading frame synthesizes three structural and seven non-structural proteins (3). It causes high temperature, muscular discomfort, headache, nausea, and rash. Symptoms of dengue bone break fever (4). Blood vessel bursts cause significant nose and mouth bleeding. Infection reduces platelets. Dengue shock syndrome causes vomiting, bleeding, and low blood pressure. Dengue hemorrhagic fever, and dengue shock syndrome kill (5).

Dengue has four serotypes. DENV-1 has six genotypes (I, II, III (sylvatic), IV, V, and VI); DENV 2 has six genotypes (Asian I, Asian II, Asian/American, American, Cosmopolitan, and sylvatic); DENV-3 has five genotypes (I, II, III, IV, and V); DENV-4 has five genotypes (I, IIA, IIB, III, and sylvatic) (6). Past studies have shown that transmission of various serotypes is cyclical, with recognizable serotypes occasionally reappearing to overwhelm and new serotypes or genotypes driving fresh scourges or episodes (3).

Dengue Critical disease is the first serotype infection. Most critical infections are asymptomatic or present as a mild febrile illness, but they may also produce hemorrhagic fever in certain patients, especially newborn infants born to DENV-invulnerable mothers. Optional dengue infection may cause dengue hemorrhagic fever (DHF) or dengue shock disorder (DSS) (7).

Dengue hemorrhagic fever affects 50 million individuals annually. Disease hurts the economy (8). This virus incubates for 3-7 days. Three stages of symptoms. High fever, lethargy, lack of appetite, vomiting, and chills. The febrile phase. In the second phase, the virus spreads methodically and causes dengue hemorrhagic fever and dengue shock syndrome, which may be fatal. The third phase is a recovery (9). Dengue hemorrhagic fever and shock syndrome have two explanations. One notion is that virulent dengue virus strains produce this illness. In another view, this is a normal dengue virus strain, but an aberrant immune response led to this disease. Dengue fever lasts 2-7 days. When a sick patient's body temperature is normal, shock occurs. Platelets drop below 100,000/mm³. Dengue hemorrhagic fever (10). Dengue shock syndrome causes increased vascular permeability, blood vessel leakage, and nosebleeds. Space fluid loses potassium and sodium ions, causing abrupt blood pressure. Due to viremia, multi-organ complications include pleural effusions, pericardial effusions, and hypotension causing mortality (11).

MATERIALS AND METHODS

Location

Lahore.

Candidate selection

The sum of 4170 candidates with Dengue fever was isolated for investigation. Each was divided by age, sex, and lifestyle. Infected and presumed dengue candidates got blood testing. Each material was tested for dengue virus IgM/IgG

antibodies and NS1 antigen using standard methods.

Sample processing

The candidate's veins were punctured to extract 2ml of blood. Blood preserved in purple EDTA tubes (K3) was added to AccuQuik™ quick diagnostic kit wells for IgG/IgM and NS1 antigen detection.

Approval

We used 4170 questionnaires as a random sample or research population. Demographic, health, previous history, occupation, and dengue fever questionnaires included fewer items. We created the questionnaire in English and translated it for those who couldn't comprehend it. As all candidates replied, they left their signatures and mobile number.

Study methods

Serology

Add Hospital name 4170 candidates' blood for dengue etiology. All tests used the AccuQuik™ kit. AccuQuik™ aims to promote global health and well-being. Through in vitro diagnostic techniques and technologies, they've launched high-quality treatments to combat malaria, dengue, Corona, and other illnesses.

AccuQuik™ IgM/IgG and NS1 antibody test kit

AccuQuik™ is a quick dengue IgG, IgM, and NS1 Antigen test kit. These tests were used to identify present or past illnesses. The gadget detects dengue fever antibodies (IgM, IgG). Detect IgM antibodies 5-8 days after exposure. IgM finding indicates early infection. The blood level will grow for a few weeks, then progressively reduce IgM antibodies after months to detectable levels. Next, IgG antibodies will be created slowly in response to infection, indicating prior infection.

AccuQuik™ Anti-NS1 Test

We utilized the widely available AccuQuik™ NS1 kit to identify dengue virus non-structural proteins NS1. NS1 is secreted in dengue-infected serum. This assay detects dengue's NS1 protein using synthetically tagged antibodies. NS1 and IgM produce diagnostic findings in 1-7 days.

CareStart™ Kit Method

The CareStart™ is a rapid diagnostic test that can simultaneously detect dengue virus in all clinical stages such as DENV-1, DENV-2, DEN-3 and DENV-4 agents. This combination of dengue DENV-1, DENV-2, DEN-3 and DENV-4 antigen allows the test to simultaneously detect dengue virus in all clinical stages. Dengue infection diagnosis, beginning with the acute stage and continuing through the convalescent stage. The presence of all four dengue serotypes in either the serum, plasma, or the entire blood of an individual. A true point-of-care test that does not require any preparation of the specimen.

Hematological analysis

Hematology analyzers can run numerous tests frequently. We used a hematology analyzer (Sysmex kx-21) for the analysis of whole blood. It is employed with 2ml of whole blood (hemoglobin, white blood cells, different leukocytes, hematocrit, and platelets). For the analysis of complete blood count, we used a purple test tube for complete blood count (CBC) which contained EDTA (K3), before the process all the samples were placed on to mixture or roller for 5 minutes at room temperature. As the mixture was done, CBC tubes were introduced to a chamber of the Sysmex kx-21 for the analysis of the blood counts.

Statistics

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 SD to find the variance, and correlation among Different groups of candidates' data based on age, gender, and Dengue serological markers like IgG/IgM and NS1.

RESULTS

The current study was conducted in Lahore, Punjab, Pakistan. As questionnaire were filled under the presence of physician hence, we proceed them for further diagnostic examination to isolate the specific antigen and antibodies against DEN-V. Study encompassed patients for diagnosis about n=450 that made account for males were 359 while females were 91, hence, among n=4820 (4170 were positive) and (65 were negative). Later, we excluded the negative

cases (77) while including positive cases (4170) only. This study included only those patients who were full bloom sign and symptoms as well as warning signs such as fever ($p = 0.007$), Rapid, weak pulse, Pallor or cool skin, Rashes ($p = 0.005$), Headache ($p = 0.001$), Joint pain,

Leukopenia, Abdominal pain, Retro-orbital pain, Persistent vomiting, Vomit with blood, Anorexia, Postural hypotension). Those Patients were excluded for diagnosis who were general fever or routine symptoms and tested negative for dengue viral infection.

TABLE 1: Questions attempted by the patients on their open choice.

Characteristics		Number (n)	Percentage (%)
Gender	Male	2856	68.4
	Female	1314	31.5
Age (Years)	15-20	1473	35.2
	21-25	870	20.8
	26-30	865	20.7
	31-35	381	9.1
	36-40	439	10.5
	40+	142	3.4
Residential area	Urban	3190	76.4
	Rural	980	23.5
Source of drinking water	Tube well	456	10.9
	River	2893	69.3
	Ponds	821	19.6
Employment status	Students	163	9.33.9
	Unemployed	525	12.5
	Tyres shop	124	2.9
	Fields	3358	80.5

TABLE 2: Questions attempted by the patients on their open choice.

Questions	Yes	No
Do you have stagnant water around your house or workplace?	78.2	21.7
Do you sleep outdoors or indoors?	68.3	31.6
Have you been diagnosed with dengue before?	12.0	88.4
Do you have previous history?	34.0	65.9

Frequency of Serological markers in candidates

The sum of $n=4170$ tested positive for dengue serological markers based on gender preference such as males were $n=2856$ (68%) and females were $n=1314$ (32%). All the serological markers (IgG, IgM and NS1) were detected by the AccuQuik™ due to its highest specificity and sensitivity rates. The collective Mean of difference was recorded for gender such as 2085 While the standard deviation was noted as 771 and Variance ($\sigma^2= 594441$) with CI 95%, $2,085 \pm 1,068.551$ ($\pm 51.25\%$). Whereas probability was calculated at 0.002.

We enlisted the clinical manifestation for dengue positive patients with severe and warm sign such as fever 4170(100%), Nose and/or gum bleeding 1321(31.6%), enlarged liver 1134 (27.1%), Skin rashes 1788 (42.8%), Vomiting/nausea 3125 (74.9%), and Body aches 2530 (60.6%). the collective Mean of difference was recorded for the severity of dengue as 2344.6 While the standard deviation was noted as 1064.47 and Variance ($\sigma^2= 1133099.22$) with CI 95%, $2,344.6667 \pm 851.754$ ($\pm 36.33\%$), Whereas probability was calculated at 0.006.

TABLE 3: Severity of dengue in positive patients based on clinical manifestation (n=4170).

Signs & Symptoms	Frequency	Percentage
Fever	4170	100%
Nose and/or gum bleeding	1321	31.6
Enlarged liver	1134	27.1
Skin rashes	1788	42.8
Vomiting/nausea	3125	74.9
Body aches	2530	60.6

The sum of n=4170 in which males were n=2856 with respect to age group such as 15-20 (1280), 21-25 (543), 26-30 (255), 31-35 (312), 36-40 (527) and 40+ (109), while females were n=1314 with respect to age group such as 15-20 (193), 20-25 (327), 25-30 (610), 30-35 (69), 35-40 (82), and 40+ (33) (table no.2). the collective Mean of difference was recorded for the Age group as 347.5 While the standard deviation was noted as 330.99 and Variance ($\sigma^2= 109560.41$) with CI 95%, $347.5 \pm 187.28 (\pm 53.89\%)$. Whereas probability was calculated at 0.001.

The sum of n=4170 in which males were n=2856 and females was n=1314. All of them tested

positive for dengue viral markers as Males were exposed to IgM=477, IgG=217, Ns1=1687, and IgG/IgM=421. Whereas females were exposed to IgM=620, IgG=318, Ns1=211, and IgG/IgM=165. The collective Mean of difference was recorded for serological markers such as IgM 548.5, IgG=294.5, Ns1=949, and IgG/IgM=293. While standard deviation was noted as IgM=71.5, IgG=23.5, Ns1=738, and IgG/IgM=128 with CI 95%, $1.960\sigma_x$ 521.25 $\pm 320.176 (\pm 61.42\%)$, the collective Variance, σ^2 for serological markers was observed as (V=213479.75). The probability of serological markers was calculated at 0.009.

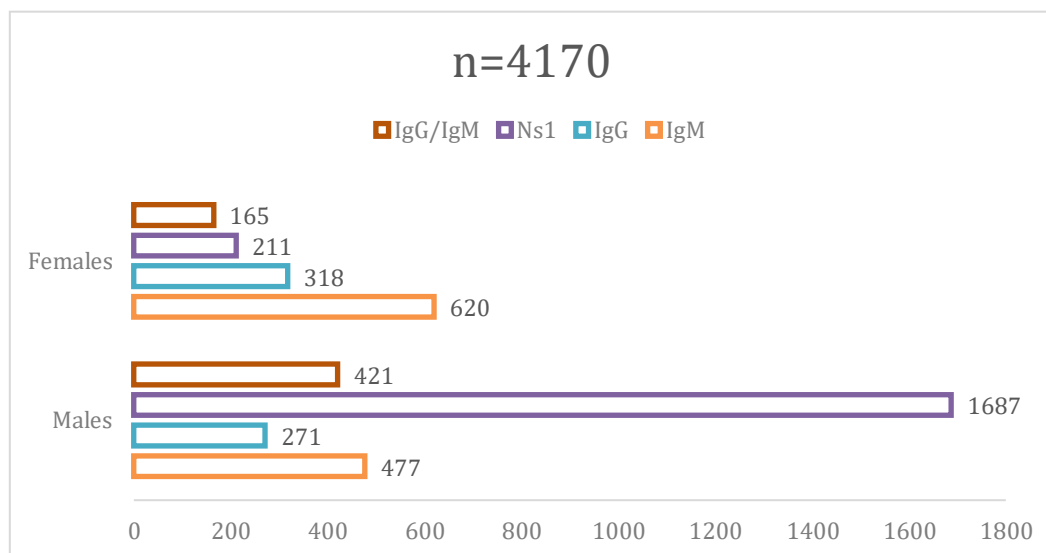


FIGURE 3: Frequency of serological markers detected in candidates (n=4170).

We used AccuQuik™ for the detection of Dengue serotypes such as DENV-1, DENV-2, DENV-3 and DENV-4 due to AccuQuik™ having good sensitivity and specificity rate. We observed dengue serotype in males like DENV-1 1337(46.8), DENV-2 521(18.2), DENV-3 653(22.8) and DENV-4 345(5.7). Whereas females created accounts like DENV-1 75(5.7),

DENV-2 410(31.2), DENV-3 283(21.5) and DENV-4 546(41.5) table no.4. The collective Mean of difference was recorded for the dengue serotypes as 521.2 While the standard deviation was noted as 350.4 and Variance ($\sigma^2= 122845.18$) with CI 95%, $521.25 \pm 242.879 (\pm 46.60\%)$. Whereas probability was calculated at 0.007.

TABLE 5: Gender-wise distribution of DENV in affected patients.

Gender	Serotypes	Frequency	%	p-value
Males	DENV-1	1337	46.8	0.007*
	DENV-2	521	18.2	
	DENV-3	653	22.8	
	DENV-4	345	12	
Females	DENV-1	75	5.7	
	DENV-2	410	31.2	
	DENV-3	283	21.5	
	DENV-4	546	41.5	

The sum of n=4170 patients was divided into two major groups based on their clinical manifestation such as dengue fever (DF) and dengue hemorrhagic fever (DHF). All patients were evaluated for the Complete blood count (CBC) through which we estimated or eradicated the affected blood parameters such as hemoglobin, platelets and hematocrits. The

Mean±SD of hemoglobin screened as of 16.0±2.41. The Mean±SD of platelets 71250.2±24321.5 and hematocrit test 47.3±7.22 in dengue fever patients. The Mean±SD of hemoglobin screened as 17.2±2.83. The Mean±SD of platelets 32412.5±27530.2 and hematocrit test 66.5±9.72 in dengue hemorrhagic fever.

TABLE 6: CBC profile for patients infected with dengue virus (n=4170).

Dengue stages	Blood parameters	Mean ± SD	CI 95%	P-value
Dengue fever	Hemoglobin	16.0±2.41	1.960	0.01
	Platelets	71250.2±24321.5		0.002**
	Hematocrits	47.3±7.22		0.005
Dengue Hemorrhagic fever	Hemoglobin	17.2±2.83		0.01
	Platelets	32412.5±27530.2		0.002**
	Hematocrits	66.5±9.72		0.007

We recorded the working status of dengue-infected candidates like Male candidates who worked Outdoor were 1721, indoor 231, and students 904. Whereas female candidates who worked Outdoor were 527, indoor 233, and students were 729. The collective Mean of difference recorded Outdoor was 1124, indoor 232, and students were 904. While standard deviation was noted as Outdoor were 597, indoor 1, and students were 175. The collective Variance, σ^2 for serological markers was observed as (V= 262200.33). We recorded the daily lifestyle status of dengue-infected candidates based on the Male candidates wearied half clothes such as 2310, full clothes 546. Whereas female candidates wore half clothes such as 253, full clothes 1061. The collective Mean of difference was recorded wearied half clothes such as 1281.5, full clothes 657. While standard deviation was noted as half clothes were 1028.5, full clothes 404, and students were 175.

The collective Variance, σ^2 for half clothes and full clothes was observed as (V= 619180.25).

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).

In this study, we focused on the only patients who tested positive for dengue viral fever or can be considered (IgG, IgM, and NS1). We found a

higher seroprevalence of dengue-like NS1 (Non-structural protein 1) across the tested population for Den-V as Males IgM=477, IgG=217, Ns1=1687, and IgG/IgM=421. Whereas female IgM=620, IgG=318, Ns1=211, and IgG/IgM=165. The Mean of difference was IgM=548.5, IgG=294.5, Ns1=949, and IgG/IgM=293. standard deviation was noted as IgM=71.5, IgG=23.5, Ns1=738, and IgG/IgM=128, CI 95%, $1.960\sigma_{\bar{x}}$, 521.25 ± 320.176 ($\pm 61.42\%$), the collective Variance, σ^2 for serological markers was observed as ($V=213479.75$). The probability of serological markers was calculated at 0.009. The highest seroprevalence of Dengue like NS1 was in males while the lowest in females due to a lot of males working in Tyre shops, Garage, students and fishermen. While a low number of cases of NS1 were reported, those females who worked outside the home, also take-care the animals and poultry. They also slept outdoors due to the warm ecosystem around their living-hood.

In a cross-sectional study of Mahmood et al., about 274 healthy populations matured within 15 years and above were arbitrarily chosen to utilize 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 dengue IgG test. Nova-Tech ELISA unit with affectability and particularity of (96.5% and 97.5%), individually, was utilized for serology (12).

Male candidates who worked Outdoor were 1721, indoor 231, and students 904. Whereas female Outdoor were 527, indoor 233, and students were 729. The Mean of difference Outdoor was 1124, indoor 232, and students were 904. While standard deviations Outdoor were 597, indoor 1, and students were 175. The Variance, σ^2 as ($V= 262200.33$). We unwrapped the highly exposed age group that increased the attention by reaching the peak of the dengue viral infection, due to these individuals working outdoors while having the issues of poverty.

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).

males with respect to age group such as 15-20 (1280), 20-25 (543), 25-30 (255), 30-35 (312), 35-40 (527) and 40+ (109), while females with respect to age group such as 15-20 (193), 20-25 (327), 25-30 (610), 30-35 (69), 35-40 (82), and 40+ (33). The most exposed age group in males was 15-20 (1280), these candidates were working outdoors, traveling from one place to another, wearing short paint and short arm shirts, and far away from schooling. Whereas the most exposed age group in females was 25-30 (610), stored water around the residential area, close interaction with water, and short/thin cloths were noted as major risk factors for dengue viral infection.

Male candidates with half clothes such as 2310, full clothes 546. Whereas female candidates with half clothes such as 253, full clothes 1061. The Mean of difference of half clothes such as 1281.5, full clothes 657. While the standard deviation of half clothes was 1028.5, full clothes 404, and students were 175. The Variance, σ^2 for half clothes and full clothes as ($V= 619180.25$).

According to Muhammad et al. Dengue seroprevalence was altogether recognized in male patients $n = 149$, 87.13%, when contrasted with female patients $n = 22$, 12.87%, $P = 0.0004$. Patients in the age group of (20–39 years, $n = 114$, 66.67%), were more inclined to dengue, however less common in the age group over (60 years). Sex, age classification, and region were critical elements in the dengue seroprevalence $P = 0.0348$. 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 bound to be IgG positive. Other danger factors related to the 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 least 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 36% (16).

Quadri et al. analyzed 378 cases among these 126 (33.3%) cases 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 patients $n = 22$, 12.87% ($P = 0.0004$). Patients in the age group of (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 favoring mosquito breeding sites (18).

CONCLUSIONS

These findings underscore the importance of considering occupational factors and daily lifestyle choices when studying dengue infection patterns. Understanding the specific environments and behaviors that contribute to dengue transmission can help in developing targeted prevention and control strategies. Further research and analysis are needed to investigate the impact of these factors in more detail and to determine the significance of other variables, such as mosquito breeding sites and individual protection measures. By gaining a comprehensive understanding of the relationship between working status, lifestyle choices, and dengue infection, public health interventions can

be better bespoke to reduce the burden of dengue and improve the overall well-being of affected populations.

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Informed Consent Statement

Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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