



DECIPHERING THE DENGUE ENIGMA: UNRAVELING CLINICAL, HEMATOLOGICAL, AND SEROLOGICAL SIGNATURES IN RAWALPINDI, PAKISTAN

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Abstract:

Dengue virus produce by the bite of the mosquito. This virus is RNA single stranded. Severe illness has the symptoms of headache, fever, joint pain and rashes. Drinking fluid can help to cure from this disease so, Recovery generally takes two to seven days. This study was carried out at District Head Quarters hospitals which is located in Punjab province, Pakistan through the duration since July 2021 to Dec 2021. Those patients who visit the DHQ hospital will be enrolled as cases while con. A cross sectional study comprised of 166 dengue patients. All the subjects were enrolled on January 2022 at District Head Quarters hospital located in Punjab province, Pakistan. Data was analyzed on SPSS IBM version22. Continuous variables are normalized by mean and standard deviation, while categorical variables are described by frequency distribution and graph pad prism help us to find the percentage of IgG, IgM and NS1. In our research we took age, Diagnoses, IgM, IgG, HCT, PLT and TLC. Some patients through suffering from dengue fever continue to develop dengue hemorrhagic

fever so in our diagnoses research we have 44.2% of DHF the study of dengue virus was divided into positive and negative according to NS1, IgM and IgG. In case of NS1 we have high rate of positive value (44.8%) and low rate of negative value (6.4%). In IgG we have 80.12% negative value and 19.88% positive value. In IgM we have 40.36% positive and 59.64% negative value. In descriptive analysis of PLT mean value is 59.29, HCT mean is 39.32 and TLC mean is 79.04. The significant of correlation of PLT and HCT is 0.05. To identify the disease, we use new molecular techniques. It has offered a fresh possibility for primary identification, even though they remain incomplete due to their expenses and standards. The use of antiviral drug medicines to cure this virus fever is still being investigated.

Key Words: Dengue virus, NS1, DHF.

Introduction

The most frequent mosquito-borne disease in mankind is dengue virus infection. Dengue virus is a parasitic sickness through a geographical distribution similar to malaria that has lately emerged as a major arboviral virus due to as urge in tropical and subtropical occurrences, as well as its increased mortality and mobility. It has a huge social impact because 2.5 billion persons living within dengue fever prone regions and are on danger of contagion on a daily basis. (Zhiyong Xi et al., 2008) RNA single stranded dengue viruses that belong to the family Flaviviridae and the species Flavivirus. Mostly they are spread to persons via the Ae Female mosquitoes, with Ae. Albopictus serving as a secondary vector. Dengue serotypes are genetically different, to each including multiple genes that show variances in infection features in mutually the human host and the mosquito vector. The incubation period of dengue mosquitoes is 7–14 days, and it varies based on the virus genotype, mosquito stain type, and environmental factors including temperature and humidity. the dengue virus initially replicates in the midgut tissue of a mosquito that consumes blood-stained dengue virus meal. (Jose L. Ramirez et al., 2008). Through the hemolymph, it then repeatedly travels to various tissues, including the larynx, body fat, and salivary glands. Peak virus titers often appear in the stomach 7–17 days after infection, and in the midgut 7–10 days. Excessive saliva and headache concentrations appear 12–18 days after feeding. (George Dimopoulos et al., 2008).

We are employing transgenesis and molecular biology methods to develop Ae species. *Aedes aegypti*'s anti- DENNV effector molecule, which triggers the RNAi response and activates dsRNA in mosquito cells. The 290 nucleotide RNA fragment from the premembrane protein coding region of the DENV2 (New Guinea C) genome, the 290 NT antisense RNA, and the intron sequence of the Ae. *Aegypti* sialokinin gene (Beerntsen et al., 1999) are present in this active RNA molecule. We demonstrated the potential effectiveness of an RNA- based strategy to stop dengue virus duplication in mosquito cells. Exposure to dsRNA from the DENV-2 genome may initiate RNAi against the subsequent DENV-2 challenge (Gaines et al., 1996, Olson et al., 2002, Adelman et al., 2001). With the use of an anti-DENNV effector chemical and the potential for an RNAi-based mosquito approach, this method has evolved into a stable, domesticated disruption approach. Transgenesis in mosquitoes is more difficult than in *Drosophila*. Using this technique, mosquitoes that produce an anti-DENV effect RNAi construct were created. The efficient use of a hairpin in cell culture procedures is the foundation for the creation of RNAi. (Kimberly M. Keene, Barry J. Beaty, Carol D., Anthony A. James, Ken E., Zach N. Adelman, Emily A. Travanty, 2004)

Replication of dengue virus:

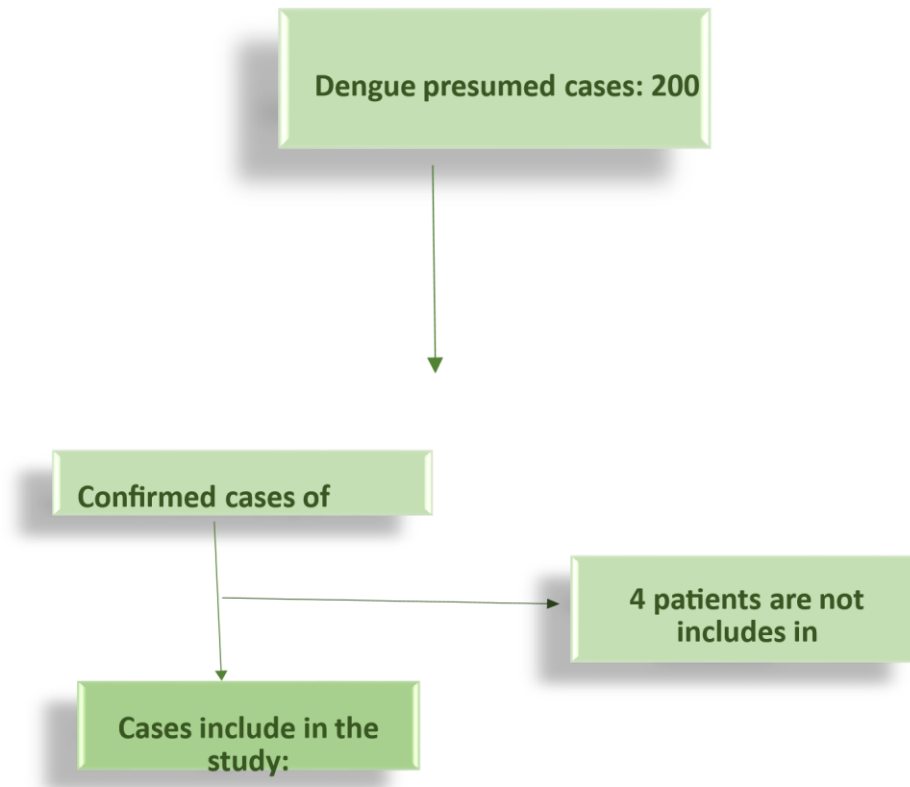
The dengue virus starts to replicate as soon as it binds to a person's skin cell. After this connection, the skin membrane encircles the virus and creates a sac around it. This sack is called an endosome.

Cells typically used endosomes to take in big chemicals and particles from their environment. The dengue virus uses this typical cellular function to its advantage in order to infect the host cell. Once inside the endosome, the virus enters the host cell and travels deep within it. The virus can still escape the endosome under two circumstances. The endosome ought to be found in an acidic environment, deep within the cell. It is necessary to preserve the endosomal membrane. In these two scenarios, the virus envelope may bind to the endosomal membrane and release the dengue nucleocapsid into the cytoplasm of the cell. A virus replicates in the cytoplasm of a cell when it is released into the cytoplasm, and the nucleocapsid splits open to display the virus's DNA. During this phase, the viral RNA is released into the cytoplasm. Eventually, the RNA of the virus overtook all of the binding cells and began to replicate. The virus uses the host's rough endoplasmic reticulum (ER) to interpret viral RNA and create the infected polypeptide through ribosomes. The polypeptides then divided into ten dengue proteins. The virus can enter the host cell and travel deep within it once it is inside the endosome. Its nucleocapsid, composed of protein C, encloses the newly generated RNA of the virus. After entering the hard ER, the nucleocapsid is encircled by the proteins M and E and has the ER membrane covering it. In this step, a viral envelope and its outer coat are generated. The immature germs pass via the complex of the Golgi apparatus, where they develop and become infectious. After that, the dengue germs are released into the cell and spread to assault other cells. (Timothy J et al., 2021).

Materials and Methods:

A total of 166 dengue patients visiting the clinic of Benazir Bhutto hospital, Rawalpindi were enrolled during September, 2021 to January 2022. A questionnaire was designed to collect information regarding biochemical, serological, hematological and clinical characteristics of the study participants.

Blood samples were collected by venipuncture technique and later on tested for the study parameters.



DATA COLLECTION METHODS AND TOOLS:

Data was collected by using a structural interviewing questionnaire, which was designed to collect and maintain all valuable information from the cases, after filling the informed consent. Specific investigations

Material required for sampling:

- Gloves
- Mask
- Tourniquet
- Antiseptic solution
- 5ml Disposable syringe
- Gel and Clot activator tubes
- Cotton

Sample collection steps:

- Assemble equipment.
- Identify and prepare the patient.
- Select the site.
- Perform hand hygiene and put on gloves.
- Disinfect the entry site.
- Take blood.
- Fill the laboratory sample tubes.

- Draw samples in the correct order.

Sample transportation:

Sample should be transported immediately to the send without any further delay. In case of delay serum should be stored at -20c

DENGUE DIAGNOSTIC METHODS:**CBC tests:**

Platelets, WBCS, RBCS and hematocrit values are commonly measured during the acute stages of dengue infection. These should be performed with care using standardized protocols, reagents and equipment Method

A drop of platelet count below 100,000 per μL may be seen in dengue fever, but this is a persistent feature of dengue hemorrhagic fever. Thrombocytopenia is usually observed between day 3 and day 8 after disease onset. Hemoconcentration, as estimated by a 20% or greater increase in hematocrit compared to convalescent values, is suggestive of hypovolemia due to increased vascular permeability and plasma leakage.

Serological methods

Until recently, the detection of dengue antigen in acute-phase serum in patients with secondary infection was rare because such patients had pre-existing virus-IgG antibody immunocomplexes. New developments in ELISA and dot blot assays directed at envelope/membrane (E/M) antigens and nonstructural protein 1 (NS1) demonstrated that high concentrations of these antigens can be detected as immune complexes in patients with both Is. Primary and secondary dengue infection up to nine days after disease onset.

MAC-ELISA method:

Total IgM in patients' sera for IgM antibody-capture enzyme-linked immunosorbent assay (MACELISA) is captured by anti- μ chain specific antibody (specific for human IgM) coated on a microplate. Dengue-specific antigens, from one to four serotypes (DEN-1, -2, -3, and -4), are bound by the captured anti-dengue IgM antibodies and directly or indirectly by monoclonal or polyclonal dengue antibodies are conjugated. An enzyme that will convert a non-colored substrate into colored products. The optical density is measured by a spectrophotometer.

Urine output:

To avoid dehydration, drink enough fluids to produce an hourly urine volume in milliliters (ml) equal to your body weight in kilograms.

To calculate the output, ensure that urine is measured at least once every four hours.

USG test:

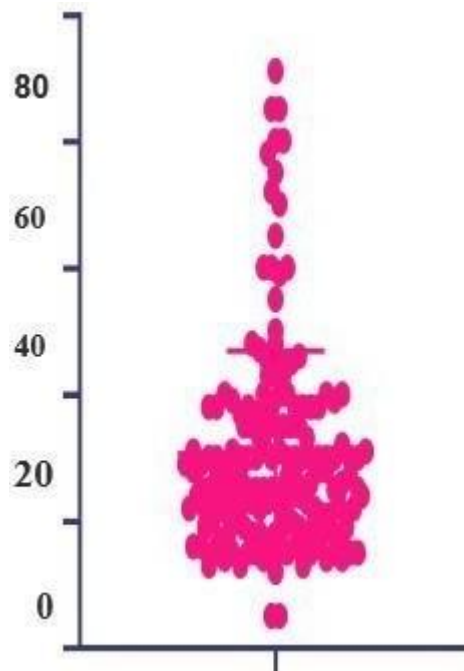
By doing Dengue Sonographic we findings may find gallbladder wall thickening, splenomegaly, pericholecystic fluid, ascites, pleural effusions, pericholecystic fluid and hepatomegaly. The presence of these findings in a suspected dengue patient provides strong support for the diagnosis, especially in endemic areas during outbreaks.

Results:

From 505,430 cases in 2000 to over 2.4 million in 2010 and 5.2 million in 2019, the number of dengue cases reported to WHO has increased by more than eight times in the last 20 years. The number of recorded deaths increased from 960 to 4032 between 2000 and 2015, with the young stage groups suffering the most. So in our research we took 166 cases, these patients are from at District Head Quarters hospitals. In our research we took age, Diagnoses, IgM, IgG, HCT, PLT and TLC. Some patients with dengue fever go on to develop dengue hemorrhagic fever so in our diagnoses research we have 44.2% of DHF the study of dengue virus was divided into positive and negative according to NS1, IgM and IgG. In case of NS1 we have high rate of positive value (44.8%) and low rate of negative value (6.4%). In IgG we have 80.12% negative value and 19.88% positive value. In IgM we have 40.36% positive and 59.64% negative value Positive IgG as well as and IgM screenings for antibodies that is present in dengue found in an initial blood sample indicate that the person was probable infected with the virus within the last few weeks. If the IgG is high but the IgM is low, the person has most certainly had an infection previously. In descriptive analysis of PLT mean value is 59.29, HCT mean is 39.32 and TLC mean is 79.04. The significant of correlation of PLT and HCT is 0.05 Ultrasonography is a simple tool for assessing suspected dengue hemorrhagic fever. If we have positive result is means that we have Pericardial effusion, thicker gallbladder wall, and fluid in the chest and abdomen. So, in our research we have highly percentage of normal USG

AGE:

Mean age= 30.6 years

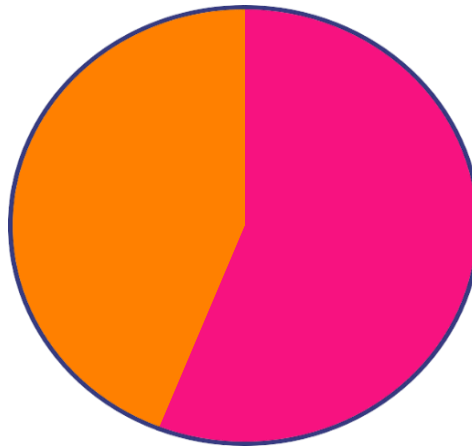


We have compiled information on the ages of the various patients. These patients are 30.6 years old on average, with a standard deviation of 16.14 years.

The average age of the patients in our sample, which is 30.6 years, is revealed by the mean age. This represents the data's core tendency.

The dispersion or spread of the ages around the mean is measured by the standard deviation (SD). It is 16.14 years in this instance. This indicates the degree to which the patient ages deviate from the

30.6-year mean. The ages are more widely dispersed from the mean when the standard deviation is larger; conversely, when the standard deviation is smaller, the ages are closer to the mean.



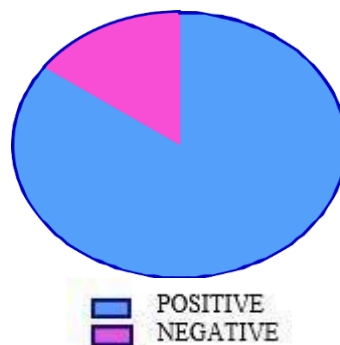
Gender Males 56%Females 44%

Males make up 56% of the dengue virus cases in our sample or community. On the other hand, women account for 44% of dengue virus cases.

The distribution of dengue virus cases by gender in our sample or population can be understood from these percentages.

Comprehending the gender-based dengue case distribution can facilitate the customization of preventive and therapeutic approaches to suit the unique requirements of various population segments.

NS1



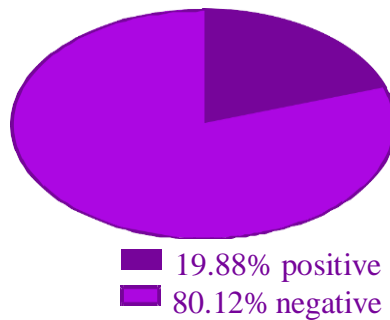
The findings of tests carried out to identify the presence of dengue virus infection are shown in this study.

The positive score, which stands for 85%, is the proportion of tests that produced a positive result for dengue virus infection. This indicates that 85% of the tests that were performed were able to detect the dengue virus. In contrast, the negative figure denotes the proportion of tests that had a negative result, meaning that dengue virus infection was not present. Thus, 15% of the tests yielded a negative result, indicating that there was no sign of dengue virus infection in those assays.

Comprehending the dispersion of dengue virus test findings yields significant understanding about the frequency and consequences of the illness, guiding public health initiatives targeted at its prevention and control.

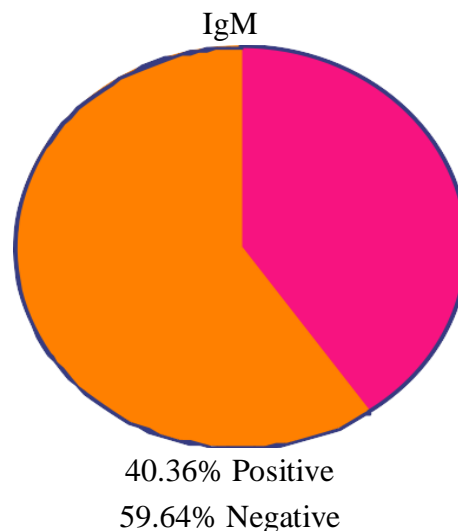
IgG

IgG



Data on IgG testing for dengue virus infection is what we are presenting. The percentage of IgG tests that produced a negative result for dengue virus infection is indicated by the negative value. The research indicates a negative value of 80.12%. This indicates that about 80.12% of the IgG tests that were performed revealed no signs of dengue virus infection. On the other hand, the positive figure denotes the proportion of IgG tests that produced a positive result, signifying the existence of antibodies against the dengue virus. The positive value in our study is 19.88%, indicating that dengue virus antibodies were detected in about 19.88% of the IgG tests. Examining IgG test results can reveal important information about the population's immunity levels and historical exposure rates.

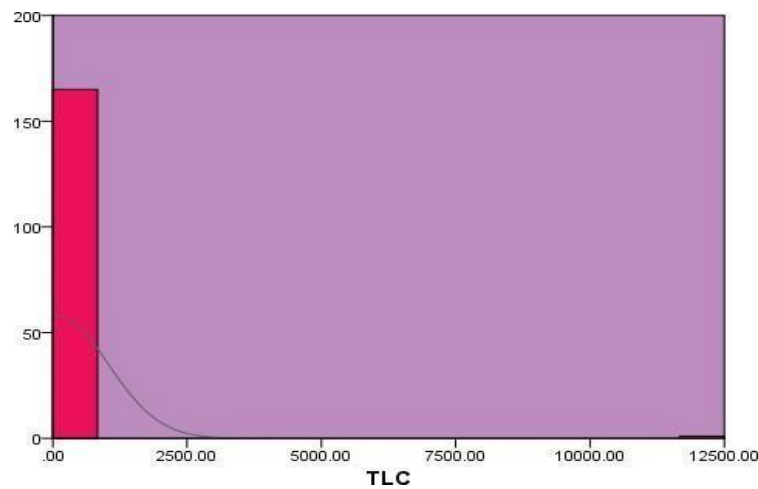
IGM



Total=166

The positive value in our study is 40.36%. This indicates that about 40.36% of the IgM tests that were performed revealed indications of a recent or ongoing dengue virus infection. On the other hand, the negative figure indicates the proportion of IgM tests that were negative, pointing to the lack of recent dengue virus infection. The negative value in our study is 59.64%, which indicates that about 59.64% of the IgM tests were unable to identify the presence of dengue virus infection. Understanding the current prevalence of dengue virus infection in the population through the analysis of IgM test results is important for developing public health policies for disease surveillance, prevention, and control.

TLC

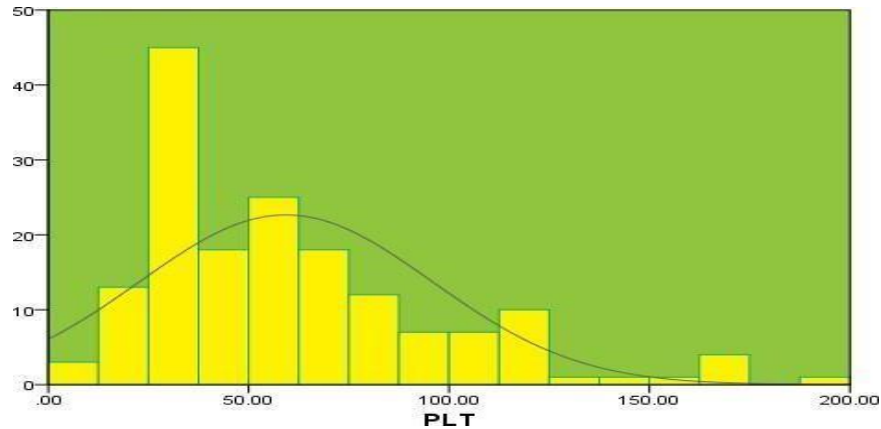


We are showcasing data pertaining to the Total Leukocyte Count (TLC) of individuals identified as having dengue fever.

The total leukocytes (white blood cells) in a microliter of blood is referred to as TLC. An essential part of the body's immunological response to infections and illnesses is played by white blood cells. The information most likely shows how dengue fever affects the total leukocyte count. In particular, compared to normal levels, there is a drop in the quantity of white blood cells in patients diagnosed with dengue fever.

Gaining knowledge on how dengue fever affects TLC can help in understanding the pathophysiology and clinical management of the illness. It emphasizes how crucial it is to routinely check test values in order to direct patient care and enhance results.

PLC



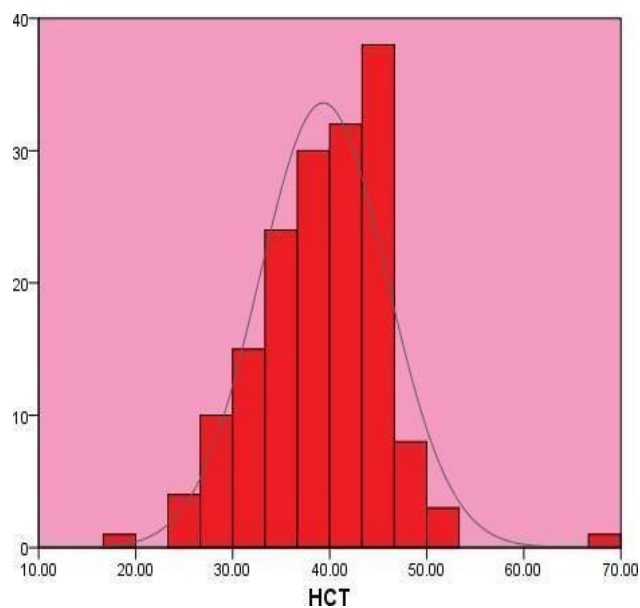
Platelet Count: Blood cells known as platelets are essential for clotting and controlling excessive bleeding. The quantity of platelets in a microliter of blood is referred to as the platelet count.

Category of Risk:

Between 40,000 and 100,000 platelets per microliter of blood is considered low risk. Individuals in this range are thought to be at little risk of developing problems from low platelet counts.

Platelet count of 21,000–40,000 per microliter of blood indicates a moderate risk. Individuals in this range are thought to be at a moderate risk of developing low platelet count-related problems. Low platelet count per microliter of blood indicates high risk. Individuals who fall below this cutoff are thought to be at a very high risk of developing problems as a result of extremely low platelet counts. Comprehending the various risk levels linked to platelet count in relation to viral infections facilitates clinical decision-making and enhances patient outcomes. It emphasises how crucial it is too frequently check the platelet count of individuals suffering from viral infections in order to avoid and treat issues linked to thrombocytopenia.

HCT



In dengue we have significant high hematocrit and have high level of hemoglobin from day 3 to day 10. When we have low level of HCT it can cause bleeding and if we have high level of HCT it can cause plasma leakage

Comprehending the dynamic fluctuations in hematocrit levels throughout dengue illness is essential for prompt diagnosis, risk assessment, and patient care. It emphasises how crucial it is to conduct careful clinical assessments and constant monitoring in order to recognize and manage any potential issues brought on by hematocrit changes.

Clinical manifestations of dengue patients during hospital visits

Clinical feature	Number of cases	Percentage
fever	148	89 %
Nausea	14	8.4 %
headache	89	53.6 %
myalgia	82	49.3 %
eye pain	38	22.5 %
mucosal bleeding	38	22.8 %
abdominal pain	61	36.7 %
nausea and vomiting	71	42.7%
Rash	13	7.8 %
torniquet test	8	4.8%
conjunctival hemmorage	23	13.8%
hepatomegaly	23	13.8%

Hematological test	No of Patient N%	Normal lab value
Platelet count		140,000-415,000
< 140,000	112 = 67%	
> 140,000	14 = 8.4 %	
WBCS Count		4,000-10500?cumm
< 4,000	40 = 24.0%	
> 4,000	80 = 48.1%	
Hemoglobin		
male < 13	30 = 18%	M: 13-16
female < 12	14 = 8.4%	F: 12-15
Hematocrit		
male >46	10 = 6%	M: 38-46
female >44	5 = 5%	F: 35-44
sub-total	15 = 9%	
Neutrophil		1500-8000
< 1500	30 = 18.07%	
> 1500	99 = 59.6%	
Lymphocytes		900-2900
< 2900	70 = 42.6%	
> 2900	49 = 29.5	

Hematological markers during hospital visit for dengue cases

parameter	no of cases	Normal lab value
ALT		3-42 IU/L
< 42	90 = 54.2%	
> 42	20 = 12%	
AST		5-37 IU/L
< 37	62 = 37%	
> 37	50 = 30%	
Creatinine		0.6 -1.1mg/dl
< 1.1	100 = 60%	
> 1.1	10 = 6%	
BUN		4.7-23.5mg/dl
< 23.5	70 = 42%	
> 23.5	11 = 6.6%	
Total protein		6.6-8.7mg/dl
< 6.6	45 = 27%	
> 6.6	80 = 48%	

Biochemical parameters among dengue cases

Descriptive and statistics analysis of biochemical profile

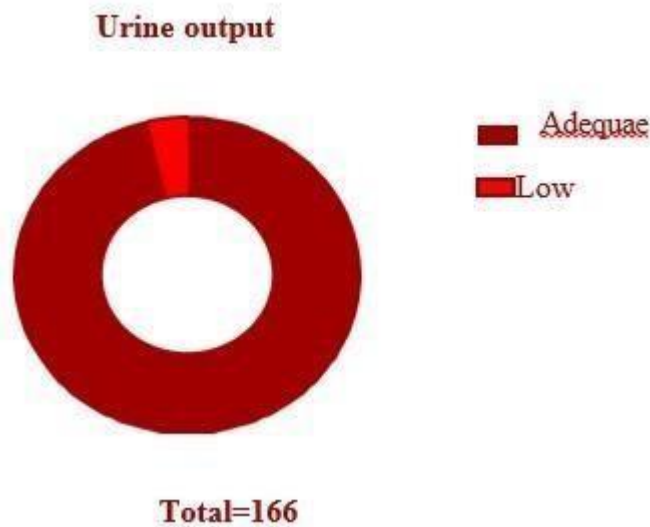
Descriptive analysis

Profile	minimum	maximum	mean	std.der
PLT	5.6	19.6	59.29	36.53
HCT	18.4	69.4	39.32	6.566
TLC	1.3	12300	79.04	954.28

Statistics Analysis:

PROFILE	PLT	HCT	TLC
Mean	59.39	39.322	79.04
Median	51.50	39.85	4.400
Mode	35.0	43.00	4.10
Std. De	36.533	6.566	954.28
Sum	9842.90	6527.60	13121.00

Urine output

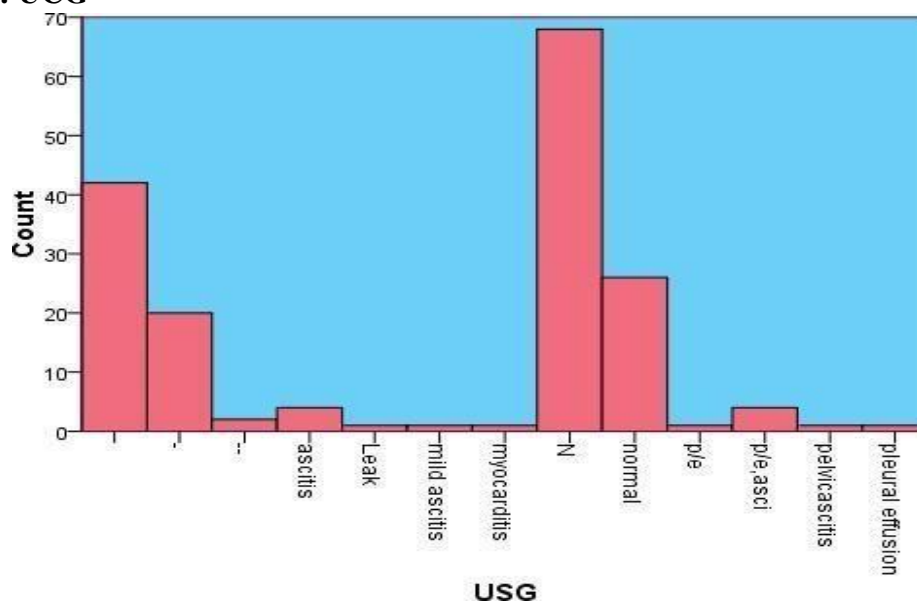


Standard Range: It is generally accepted that a 24-hour urine output of between 800 and 2,000 millilitres per day is the usual range. This range guarantees the body's fluid balance and appropriate kidney function.

High Adequate Output Percentage (92.4%): According to the statistics, 92.4% of the study population had a 24-hour urine volume that was within the typical range of 800 to 2,000 millilitres per day. This represents a major portion of the population.

It is crucial to comprehend and keep an eye on urine production when evaluating renal function, fluid balance, and general health. The information shown in Figure 5.9 offers important insights about the sufficiency of urine flow in the study population and can inform clinical decision-making and patient care practices.

Fig 5.10: USG



The information indicates that almost all of the study's patients had normal ultrasonography (USG) results. Let's look at what this finding means:

Ultrasonography: USG is a non-invasive imaging method that produces images of the body's internal structures by using high-frequency sound waves. Assessing different organs and systems, such as the abdomen, pelvis, and cardiovascular system, is a popular usage for it. Typical Results: For the vast majority of the patients in the research, the data shows that the USG results were within normal limits. This implies that during the imaging evaluations, no notable anomalies or disease were found. Gaining knowledge about the frequency of typical USG results in the research group can help determine how useful USG is as a diagnostic tool in both clinical and research contexts. It emphasises how crucial it is to consider the clinical history, symptoms, and other diagnostic findings when interpreting imaging results in order to guarantee a precise diagnosis and the best course of treatment

Discussion

In Pakistan dengue fever is becoming more common. It is a new disease as compared to malaria, therefore it is very important to analyze so we can easily prevent the effect of dengue disease from our discussion we have to conclude to find out the results and factors responsible for its spread and we also formulate future strategies to control it Pakistan is said to have the highest prevalence of dengue in 2019 and the population suffering from this disease is said to be between 30,000 or above. Even we don't have specific medicines to treat dengue for reducing fever we mostly use acetaminophen. We should take rest, drink plenty water or any kind of fluids to cure acute dengue disease. The ideal properties of taking fluid are stabilizing the heartbeat and carrying nutrients and oxygen to your cells. Patients with HF may require intravenous volume replacement so it can prevent nausea and vomiting. In this study, among 166 dengue cases, some patients have symptoms of dengue but have negative results. So, it means that patients are not suffering from dengue virus. The tendency of dengue occurs mostly in both younger and older age group and we also have equal rate of male and female. Our Studies have also shown varying incidence in NS1, IgG and IgM, so we have highly percentage of NS1 In our research we came to know that male suffer most then female because male have strong interaction with the environment and social areas, due to this reason the male percentage will be high almost 56% male and 44% female Our observational study done on 166 patients showed that the initial response of fluid intake and acetaminophen drugs had excellent response rates because this drug reduce fever. DF is an acute febrile disease and it's about 5.9% however DHF is more severe form of disease which can affects blood and lymph vessels. in our research it has high percentage of about 44.6% and DSS is so rare it's about 0.2% Tests for the dengue virus's non-structural protein, NS1, are linked to the virus. When dengue is present, this protein is discharged into the bloodstream. Serum-based NS1 assays have been developed. To diagnosis fever, particularly if it is experienced, the Dengue Antigen NS1, IgG, and IgM test is performed. The percentage of negative results from our sample of NS1 is 15% and the positive result is 85%. So according to IgG we have 80.12% negative value and 19.88% positive 53 value and in IgM we have 40.36% positive and 59.64% negative values so according to our research we should avoid aspirin medicine There is a decrease in white blood cell and platelet counts in cases of dengue fever. In healthy individuals, the platelet count ranges from 1.5 to 4 lacs; in dengue sufferers, it can drop as low as 20,000 to 40,000. The standard deviation of PLT is 36.53, while its mean value is 59.29. The Hematocrit Test (HLT) is it. From day 3 to day 10, the dengue patient had higher hemoglobin and hematocrit levels than the control group. The majority of patients had normal white blood cell counts in the early stages of dengue fever, with a mean value of 39.32 and a standard deviation of 6.56. Any alteration in the total leucocyte count results indicates that the condition is becoming more severe. The majority of dengue fever patients have neutropenia. Thus, the standard deviation is 954.28 and the mean value is 79.04. An estimated 400 million people are thought to contract the dengue virus each year via mosquito bites. Wearing long sleeves, using insect repellent, and controlling mosquitoes both inside and outside the home are

all important steps towards eradicating this illness. Antiviral medications may be able to treat dengue if we are infected with this virus; this is still being researched.

Conclusion

Currently, the dengue virus is a global issue. New molecular-based diagnostic techniques have created a new window of opportunity for early diagnosis, but standards and costs continue to be barriers. Research is still ongoing regarding the use of antiviral medications to treat dengue fever. We should take the following precautions to avoid contracting the dengue.

- Take precautions against mosquito bites.
- Keep mosquitos at bay both in and out of your home
- Avoid going to mosquitoinfested places.
- Apply a mosquito repellent.
- Protect your arms and legs by wearing long sleeves and long pants.
- While sleeping, use mosquito netting

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