IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
March 2023 Vol.:26, Issue:4

Diagnosing the Correlation of NS1 Antigen Titres with the Severity of Dengue Fever in Children

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Gowtham Mandadapu[1], Prachetha Kolli[2], J N Suresh Kumar[3], Kurra Venkata Gopaiah[3*]

1. Managing Director, Devansh Lab Werks Inc 234 Aquarius Drive, Suite111, Homewood, Alabama-35209.

2. Managing Director, Microgen Health, 14225 Sully field Circle, Suite E, Chantilly, VA- 20151.

3. Professor & Principal, Narasaraopeta Institute of Pharmaceutical Sciences, Kotappakonda Rd, Yellamanda, Narasaraopeta, Andhra Pradesh, India-522601.

4. Associate Professor, Narasaraopeta Institute of Pharmaceutical Sciences, Kotappakonda Rd, Yellamanda, Narasaraopeta, Andhra Pradesh, India-522601.

Submitted: 22 February 2023
Accepted: 28 February 2023
Published: 30 March 2023

HUMAN JOURNALS
www.ijppr.humanjournals.com

Keywords: NSI, ELISA, Antigen, Morality.

ABSTRACT

In present study we can conclude that NS1 Antigen ELISA titers may not be useful in predicting the severity of dengue fever in children and further research is warranted for early identification of severity of dengue which can help in significant reduction in mortality. However, our study had few limitations that NS1 antigen Assay was not quantitated and NS1 Assay was not performed uniformly on one particular day of illness which would have warranted more clinical significance. As per the discussion Dengue fever is a recently emerging health problem with increasing epidemics every year. The revised World Health Organization dengue fever guidelines 2011 have stressed the need for early diagnosis and treatment to reduce the mortality due to severe dengue infection. However, there are no proven or clear interventions or modalities to predict the severity of dengue fever in children. In present study we have aimed at developing a predictor of severity of dengue fever. This is a cross sectional study which was conducted in a tertiary care teaching. We have aimed to find out the association between NS1 Antigen titer levels and the severity of dengue fever in children between January 2022 to Feb 2023. NS1 titer levels were measured using ELISA method and it was correlated with Clinical severity.

INTRODUCTION

Dengue infections are currently one of the most rapidly emerging arboviral infections in the world, which result in 390 million infections every year.[1] They cause significant morbidity and mortality especially in developing countries and is a huge burden on their economies. Although the majority of dengue infections result in asymptomatic infection or manifest as undifferentiated viral fever, some develop fluid leakage and bleeding manifestations which result in dengue hemorrhagic fever (DHF) and dengueshock syndrome (DSS.) As there is no effective antiviral treatment or a licensed vaccine to prevent infection, meticulous fluid management and monitoring for complications is currently the only option available. Earlier case fatalities due to dengue infection have been reported to be around 2.5% to 5.4%.[1] Shock and organ impairment have been shown to be the most important factors that lead to fatalities in dengue infection.

As a result of better fluid management regimens and greater awareness of associations of severe dengue and early interventions, the case fatality rates have significantly dropped in many dengue endemic countries. However, in order for early detection of those who are likely to develop severe dengue, the clinical and laboratory parameters are measured at least two or three times a day in all patients admitted to the hospital with dengue infection.

Although ideal management of children includes monitoring of many clinical parameters at least every 2 hours, this is sometimes impossible due to limited health resources. Therefore, a simple test that can be done in a ward would be of upmost importance to determine the children who are most likely to develop severe clinical disease. Early diagnosis and prompt treatment can help in reducing significant mortality and our study has attempted to identify a predictor of severe dengue.

Dengue viruses originates from animal reservoir. Two distinct DENV transmission cycles are recognized.

- Endemic/epidemic cycle
- Sylvatic /zoonotic cycle

Endemic and epidemic cycles involve the human host and viruses are transmitted by *A. aegypti, A. Albopictus* and other mosquitoes as secondary vectors. The sylvatic transmission

cycle involves monkeys and several different Aedes mosquitoes identified in Asia and West Africa.

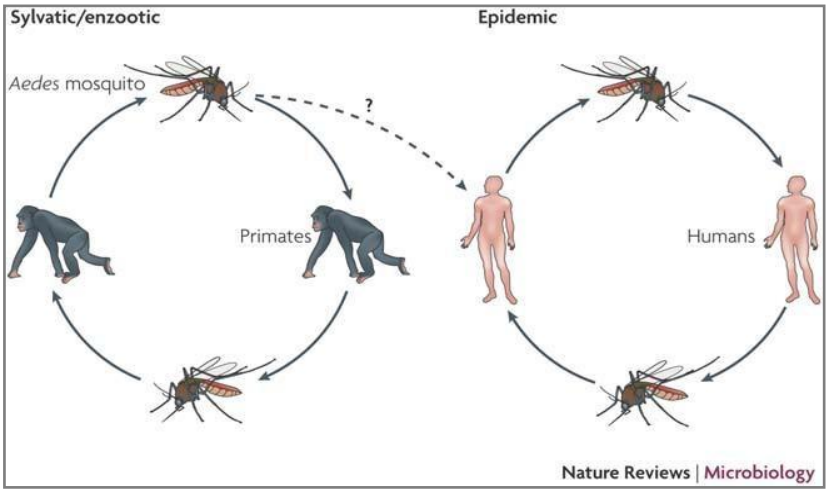

Figure No.1: The two DENV transmission cycles

To clearly understand the real burden of the disease the geographical location of the disease should be studied in detail. The dengue infection has more prevalence in the tropical region and many of the areas that are categorized as high-risk areas come under Asia. Temperature of a particular region plays a major role in the survival of the vector which is the major mode of transmission. The socioeconomic status also aids with the spread of infection. India was challenged with the first epidemic in Chennai and later in Calcutta which was proven virologically in the year of 1963. Delhi the most predominant type of dengue virus was of type 2 and 3 but recent trends show type 1 as the most prevalent type in the city of Delhi. Dengue is mostly spread by the bite of day time mosquitos in contrast to other disease vectors. Stagnation of clean water is the suitable place for breeding of these vectors. Individuals once exposed to a mosquito bite the virus enters the body through systemic circulation and lodges in the lymphatics and from there gets disseminated to various organ systems of the body. It affects many systems of the body but most significant among them are reticuloendothelial system, endothelial cell lining the various blood vessels in the body. The exact mechanism by which there is varied clinical spectrum of the same disease is not understood clearly, but through various studies though not fully much more information regarding the pathogenic aspect of the disease has been tried to figure out recently. Hypothesis had been stated that the imbalance between the inflammatory cascade which is triggered by the infection and anti-inflammatory pathway is responsible for the disease to manifest in various severity levels. Infection leads to increased production of inflammatory mediators and increased destruction of endothelial cells

leading to the destruction of platelets all of leading to plasma leakage which holds responsible for most of the clinical manifestations of the clinical spectrum which ranges from mild disease to deadly severeform of the disease can be classified broadly into:

NON-SEVERE FORM

- Asymptomatic infection
- Classical dengue fever

SEVERE FORM

- Dengue hemorrhagic fever
- Dengue shock syndrome

CLINICAL FEATURES:

Common symptoms

- Fever
- Headache, arthralgia, malaise, fatigue
- Generalized maculopapular rash – sometimes associated with itching
- Facial flushing
- Conjunctival congestion
- Gum bleeding

MATERIALS AND METHODS

STUDY DESIGN: Hospital based prospective observational study

PLACE OF STUDY: PSG Hospitals, PSGIMS&R, Coimbatore

TIME PERIOD: January 2022 to Feb 2023

SAMPLE SIZE: 250 samples were required based on the formula 4p9/d2 considering the prevalence of dengue fever.

INCLUSION CRITERIA:

- Age: less than 18 years
- Study was carried out in children with clinical suspicion of dengue feverwith features like:
- Fever > 3 days
- Myalgia
- Rash
- Arthralgia

EXCLUSION CRITERIA

- Children with dengue fever and co infections were excluded.

METHODOLOGY

Ethical committee approval was obtained and Prospective collection of data was done in children who fulfilled the inclusion criteria in the Pediatrics department at tertiary care center, PSGIMSR, Coimbatore where systematic computer coding for registry is used. NS1 Antigen ELISA was done in all children between day 1 to 5 illness who fulfilled the inclusion criteria and 2ml of blood was drawn and collected in EDTA container. NS1 Antigen ELISA wastested using (J-Mithra kit) India at the Serology lab in our hospital. Qualitative and semi-Quantitative analysis was done.

Titer of >11 was considered strongly positive, 9-11 as equivocal and titer value of 9 was mild positive. Dengue Serology for IgG antibodies for secondary infection and IgM antibodies for primary infection were performed on the same children using ELISA Assay. All other relevant and additional investigations weredone as per the course of illness.

Data was entered in structured proforma and case definition, diagnosis and management used for dengue fever were categorized into Mild, Moderate and Severe dengue as per revised NVBDCP guidelines. All other relevant clinical data were collected.

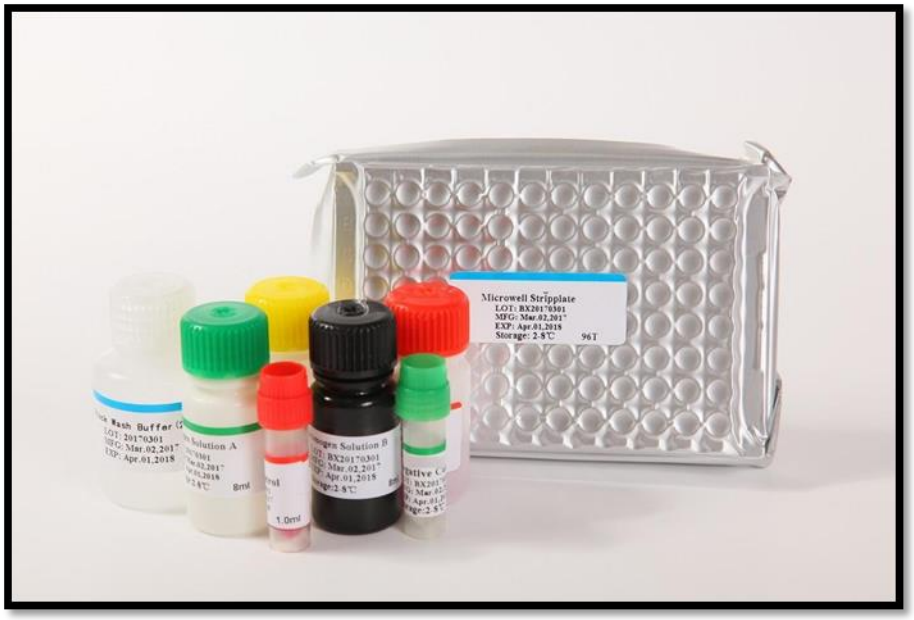

Figure No.2: Shows the J Mithra kit used for estimating NS1 Antigen ELISATiters

Statistical Tools:

The data collected from the patients were tabulated using Microsoft Excel. The SPSS 23 statistical software was used for data analysis. After collecting all the data, all the variables were summarized by descriptive statistics. Categorical variables were expressed as frequencies and percentages, and Descriptive analysis was done using chi-square test and p value of <0.05 wasconsidered statistically significant.

STRUCTURAL IMPORTANCE OF DENGUE VIRUS

Dengue virus is a positive stranded, encapsulated RNA virus 11kb in sizeand has a single Open Reading Frame (ORF) encoding for a single polypeptide which is further processed into three structural proteins that is the Capsid (C), Membrane (M), and Envelope (E) proteins, and seven non-structural (NS1) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5).

Seven nonstructural proteins: NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. These nonstructural proteins play roles in viral replication and assembly.

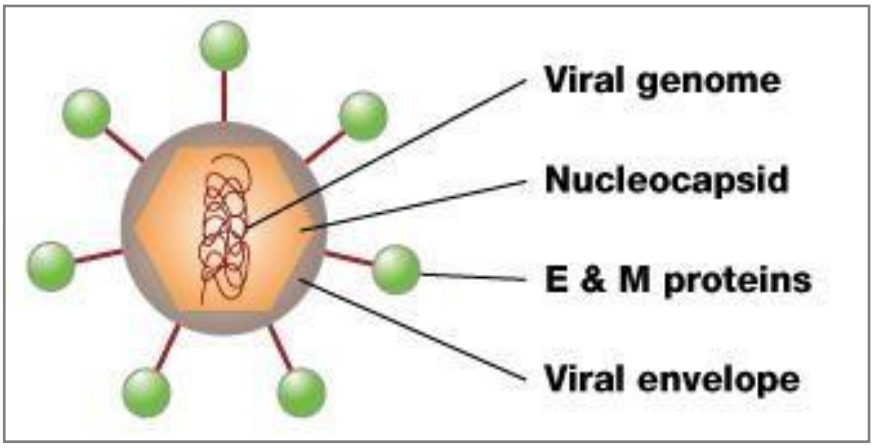

Figure No.3: Dengue virus structure

The dengue virus has a roughly spherical shape. Inside the virus is the nucleocapsid, which is made of the viral genome and C proteins. The nucleocapsid is surrounded by a membrane called the viral envelope, a lipid bilayer that is taken from the host. Embedded in the viral envelope are E and M proteins that span through the lipid bilayer. These proteins form a protective outer layer that controls the entry of the virus into human cells.

The structure of the dengue virus is roughly spherical, with a diameter of approximately 50 nm (1 nm is one millionth of 1 mm). The core of the virus is the nucleocapsid, a structure that is made of the viral genome along with C proteins. The nucleocapsid is surrounded by a membrane called the viral envelope, a lipid bilayer that is taken from the host. Embedded in the viral envelope are 180 copies of the E and M proteins that span through the lipid bilayer. These proteins form a protective outer layer that controls the entry of the virus into human cells.

Dengue Virus Replication and Infectious Cycle

The dengue viral replication process begins when the virus attaches to a human skin cell. After this attachment, the skin cell's membrane folds around the virus and forms a pouch that seals around the virus particle. This pouch is called anendosome. A cell normally uses endosomes to take in large molecules and particles from outside the cell for nourishment. By hijacking this normal cell process, the dengue virus is able to enter a host cell.

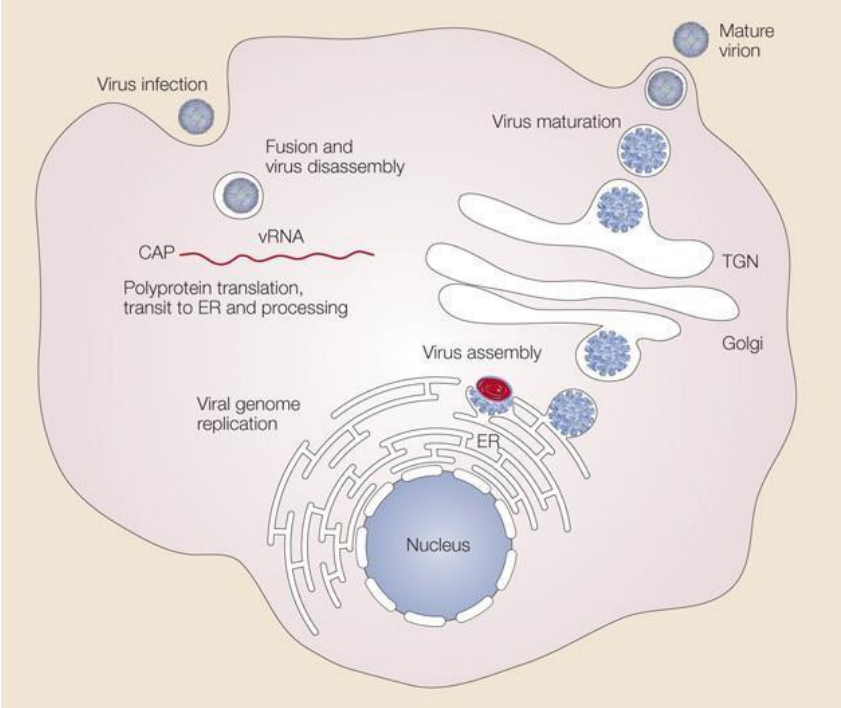

Figure No.4: Dengue virus replication

The dengue virus attaches to the surface of a host cell and enters the cell by a process called endocytosis. Once deep inside the cell, the virus fuses with the endosomal membrane and is released into the cytoplasm. The virus particle comes apart, releasing the viral genome. The viral RNA (vRNA) is translated into a single polypeptide that is cut into ten proteins, and the viral genome is replicated. Virus assembly occurs on the surface of the endoplasmic reticulum (ER) when the structural proteins and newly synthesized RNA bud out from the ER. The immature viral particles are transported through the trans-Golgi network (TGN), where they mature and convert to their infectious form. The mature viruses are then released from the cell and can go on to infect other cells. Once the virus has entered a host cell, the virus penetrates deeper into the cell while still inside the endosome.

1. The endosome must be deep inside the cell where the environment is acidic.

2. The endosomal membrane must gain a negative charge.

These two conditions allow the virus envelope to fuse with the endosomal membrane, and that process releases the dengue nucleocapsid into the cytoplasm of the cell.

Once it is released into the cell cytoplasm, the nucleocapsid opens to uncoated the viral genome. This process releases the viral RNA into the cytoplasm. The viral RNA then hijacks

the host cell's machinery to replicate itself. The virus uses ribosomes on the host's rough endoplasmic reticulum (ER) to translate the viral RNA and produce the viral polypeptide. This polypeptide is then cut to form the ten dengue proteins.

The newly synthesized viral RNA is enclosed in the C proteins, forming a nucleocapsid. The nucleocapsid enters the rough ER and is enveloped in the ER membrane and surrounded by the M and E proteins. This step adds the viral envelope and protective outer layer. The immature viruses travel through the Golgi apparatus complex, where the viruses mature and convert into their infectious form. The mature dengue viruses are then released from the cell and can go on to infect other cells.

There are four serotypes of dengue virus (DEN1-4) and the recovery of infection differs from one serotype can confer life-long protection against that serotype but not against the other three serotypes. Severe dengue infection usually occurs after a second infection with a different serotype, which is due to immune-mediated antibody-dependent enhancement (ADE). The revised World Health Organization dengue fever guidelines 2011[25] have emphasized the need for early diagnosis and treatment to reduce the mortality due to severe dengue infection. The classical methods of confirmation of diagnosis are virus isolation, serotype identification, antibody detection tests (IgM and IgG MAC-ELISA), hemagglutination inhibition or neutralization tests but all these tests are time consuming and do not help in the confirmation of diagnosis.

SIGNIFICANCE OF NS1 ANTIGEN

The NS1 protein is a glycoprotein that is produced in infected cells, but is not incorporated into the virion. In a child with dengue, NS1 is situated on the plasma membranes of cells and in the circulation. Antibodies interact with NS1 to cause complement-dependent lysis of virus-infected cells. Further NS1-specific antibodies probably contribute to antibody-dependent cellular cytotoxicity. Neutralization of infection by dengue virus-specific antibodies can occur through several different mechanisms, including inhibition of binding to cell surface receptors or post-binding inhibition of viral fusion with endosomes. Neutralizing antibodies directed against the E protein are highly serotype cross-reactive.

The tropism of dengue virus for monocytes and macrophages, which both express receptors for immunoglobulins, leads to the entry of dengue virus into host cells, this phenomena is called “antibody-dependent enhancement of infection”.

This occurs because virus-antibody complexes infect host monocytes more efficiently than free virus particles. Anti-body dependent enhancement of infection can be mediated by E protein-specific or pre-M antibodies and occurs when antibody concentration is low so that the number of antibody molecules bound per virion is below the threshold necessary for neutralization of the virus. In genetically predisposed individuals, subsequent infection of monocytes by virus-antibody complexes can also influence cellular immune responses.

NS1 ELISA TESTING

Enzyme-Linked Immunosorbent Assays (ELISA) directed against Non-Structural glycoproteins (NS1 Antigen) have demonstrated very high concentrations in the sera of dengue virus infected patients during the early clinical phase of the disease and represents a new approach to the diagnosis of acute dengue infection.

NS1 antigen assay, there has shown marked rise in early diagnosis of dengue fever during the first week of illness especially during epidemics but its role as an early predictor of severe dengue infection is not very clear. The major diagnostic methods currently available are viral culture, viral RNA detection by reverse transcriptase PCR (RT-PCR) and serological tests such as an immunoglobulin M (IgM) capture enzyme-linked immunosorbent assay (MAC-ELISA). However, early dengue diagnosis still remains a problem, as all these assays have their own draw backs. The first two assays have restricted scope as a routine diagnostic procedure. Viral isolation by cell culture and subsequent detection by immune fluorescence, though the gold standard cannot be used as a routine diagnostic procedure due to its low sensitivity and time consumption. The requirement of a highly trained staff, the need of a sophisticated equipment as well as the cost factor associated with molecular methods has limited its application as a routine diagnostic assay. The requirement of paired sera at acute and convalescent phase, which improves the accuracy of the diagnosis, further delays the treatment. NS1 (non-structural protein 1) is a highly conserved glycoprotein that is essential for the viability of DV and is produced both in membrane-associated and secretory forms by the viruses.

Enzyme-linked immunosorbent assays (ELISA) directed against NS1 antigen (NS1 Ag) have demonstrated its presence at high concentrations in the sera of DV infected patients during the early clinical phase of the disease.

RESULTS

Our study was conducted at Pediatrics department PSG Hospitals, Coimbatore which is a tertiary care hospital. Our study period was between January 2016 to May 2017. In a total of 690 children who were serologically positive for Dengue fever, 270 children were included in the study and 250 children were finally included for study analysis and 20 children were excluded because of logistic reasons and comorbidities. Results were tabulated and comparisons were done.

Table No.1: Distribution of study population according to age group

Age group	Number	Percentage
< 5 years	120	48
6-10 years	71	28.4
11-15 years	40	16
>15 years	19	7.6
Total	**250**	**100**

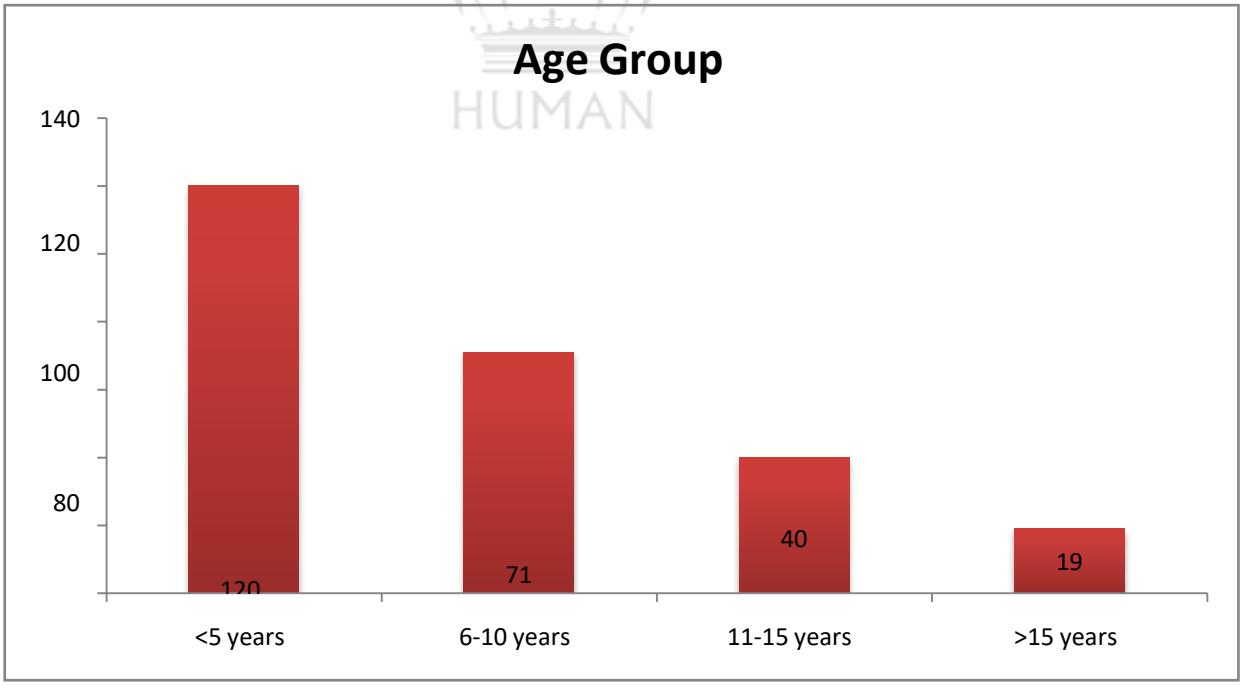

Figure No.5: Distribution of study population according to age group

The total number of 250 children included in the study. Out of 250 children 120 children (48%) were less than 5 years, 71 children (28.4%) were between 6 to 10 years, 40 children(16%) were between 11 and 19 children (7.6%) were above 15 years.

Sex Distribution

Study Group	Male	Female
Number	133	117
Percentage	53%	47%

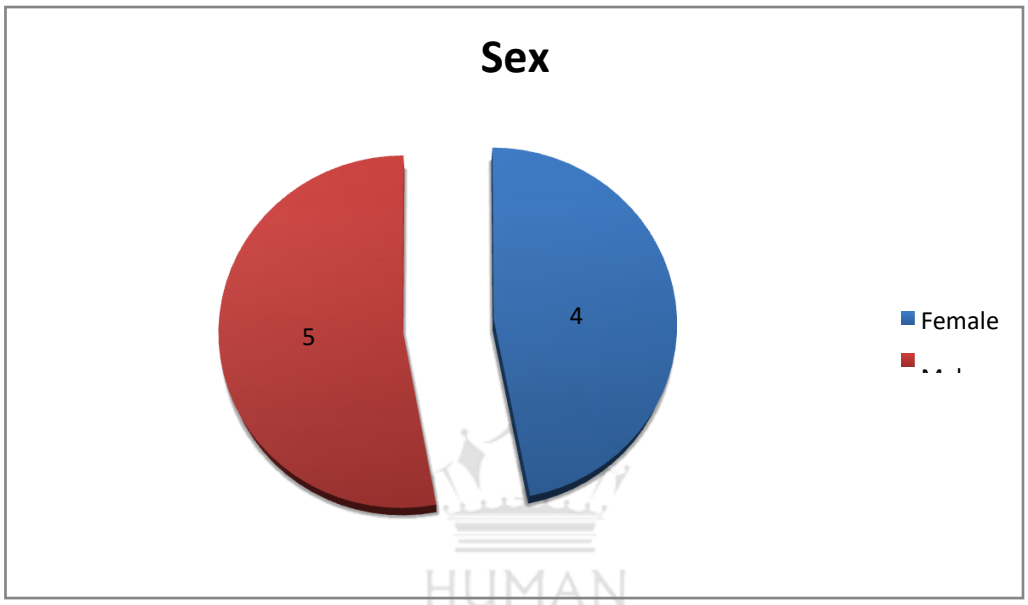

Figure No.6: Sex distribution of the study group of which 53% were male children and 47% were female children.

Table No.2: Distribution of study population according to admission status

Admission	Number	Percentage
Admitted	224	89.6
Not admitted	26	10.4
Total	**250**	**100**

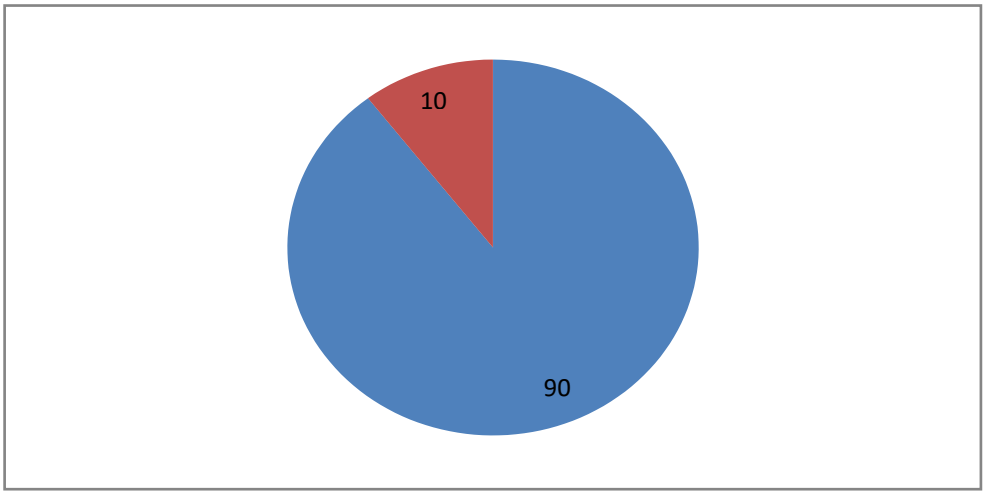

Figure No.7: Distribution of study population according to admission status

Admission status of study population. Out of 250 children 224 children were admitted (90%) and 24 children (10%) were treated as outpatients.

Table No.3: NS1 Antigen positivity related to day of illness

Day of illness	Number	Percentage
≤3 days	158	63.2
4 to 5 days	92	36.8
Total	**250**	**100**

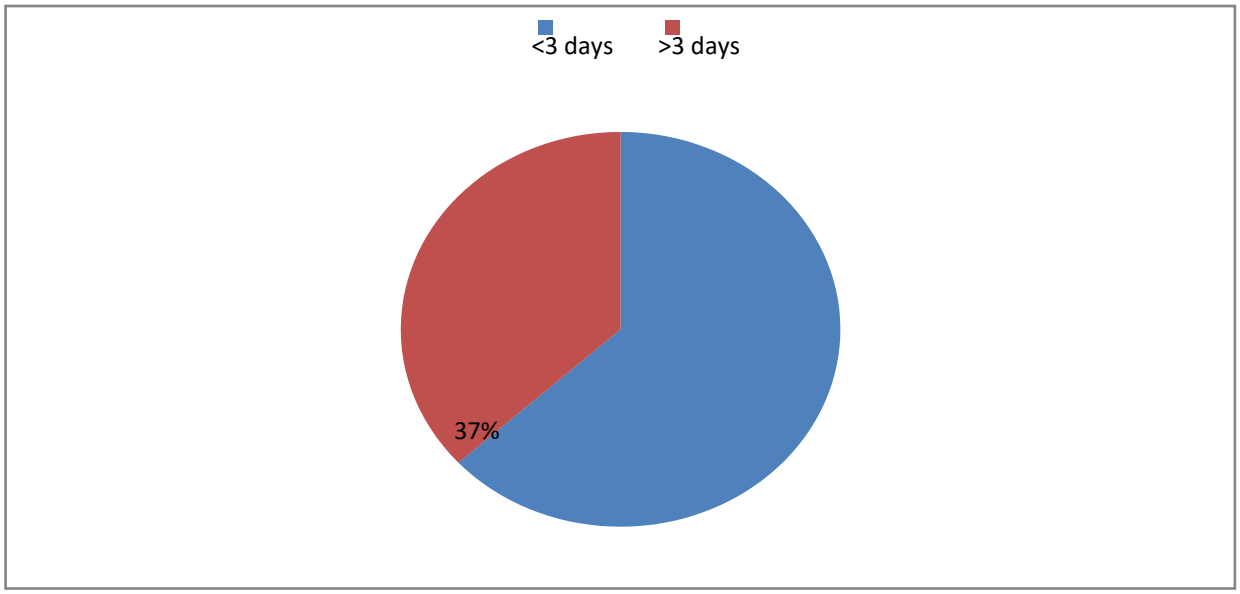

Figure No.8: NS1 Antigen positivity related to day of illness

That NS1 titer values were found to be positive in 158(63.2%) children when NS1 antigen Assay was done in less than 3 days of illness. NS1 antigen was positive in 92 children (36.8%) when done on 4 to 5 daysof illness.

Table No.4: Distribution of study population according to NS1 titer levels

NS1 levels	Number	Percentage
< 9	81	32.4
9-11	74	29.4
>11	95	38
Total	**250**	**100**

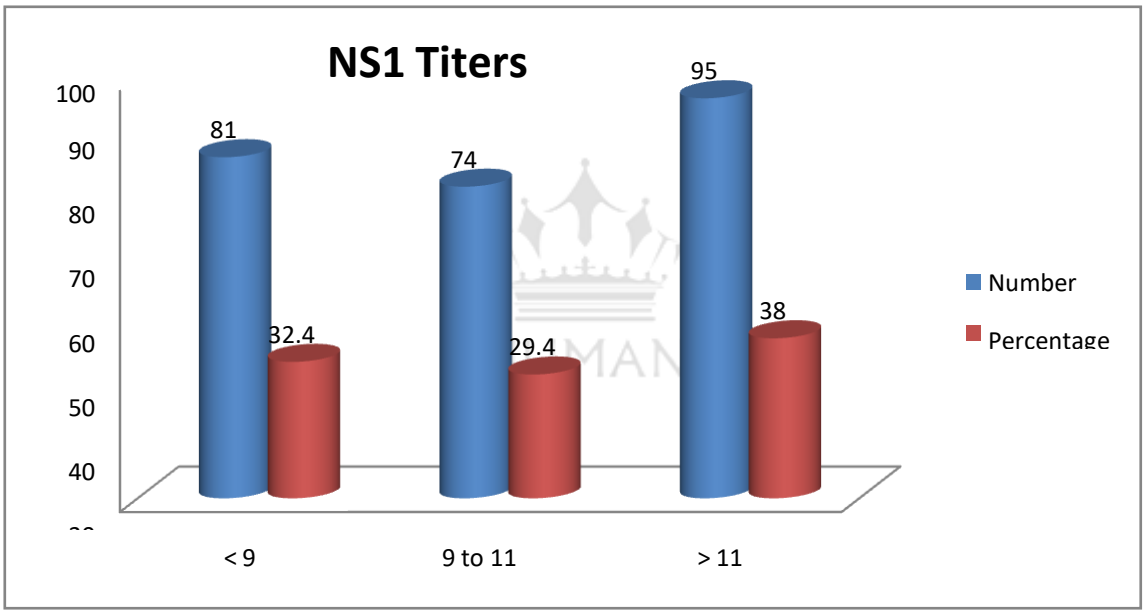

Figure No.9: Distribution of study population according to NS1 titre levels

The titre values of the study population. Titre values of <9 were seen in 81(32.4%) children, titre values of 9 to 11(29.4%) were seen in 74children and titre values of >11(38%) were seen in 95(38%) of the children.

Table No.5: Association between age group and NS1 titre levels in the study population

Age group	Titres<9	Percentage
< 5 years	43	35.8
6 - 10 years	23	32.4
11 - 15 years	8	20
>15 years	7	36.8

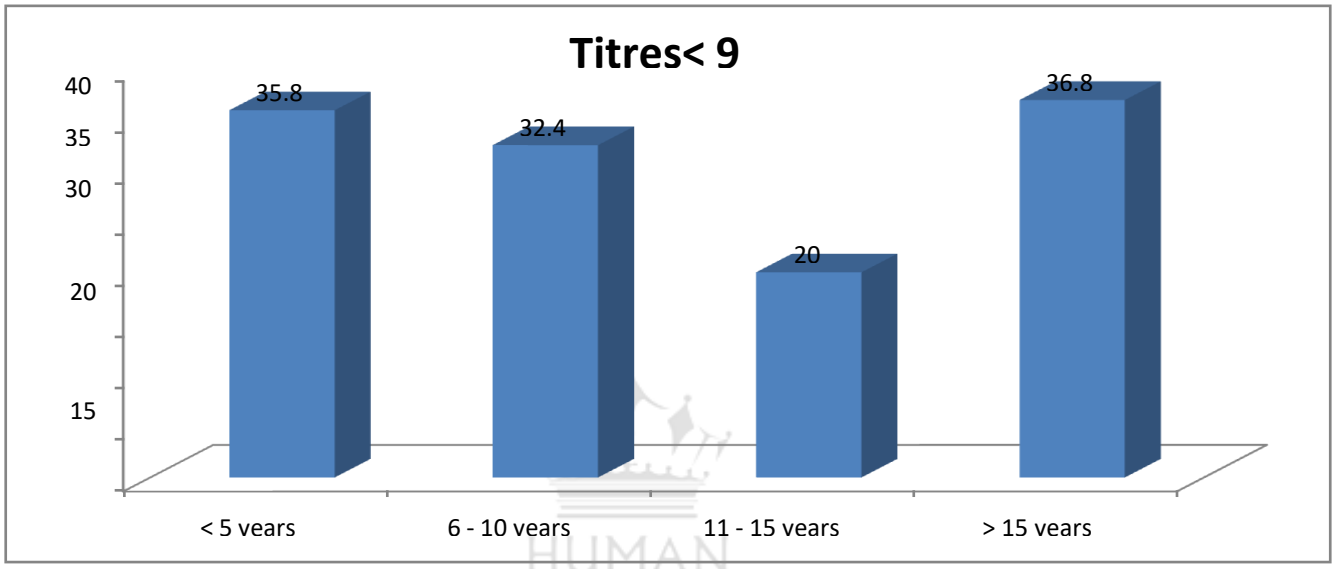

Figure No.10: Association between age group and NS1 titre levels in the studypopulation

The association between age group and and NS1 titres. In age group of less than 5 years, titre values of less than9 were seen in 43 children (35.8%) and in age group of 6 to 10 years titre values of less than 9 was seen in 23 children (32.4%). In children with age group of 11 to 15 years titre values of <9 was seen in 8 (20%) children. Children with age group of 11 to 15 years NS1 antigen titre values of <9 was seen in 7(36.8%) children.

Table No.6: Association between age group and NS1 titre levels in the study population

Age Group	Titres 9 to 11	Percentage
< 5 years	32	26.7
6 - 10 years	26	36.6
11 - 15 years	12	30
> 15 years	4	21.1

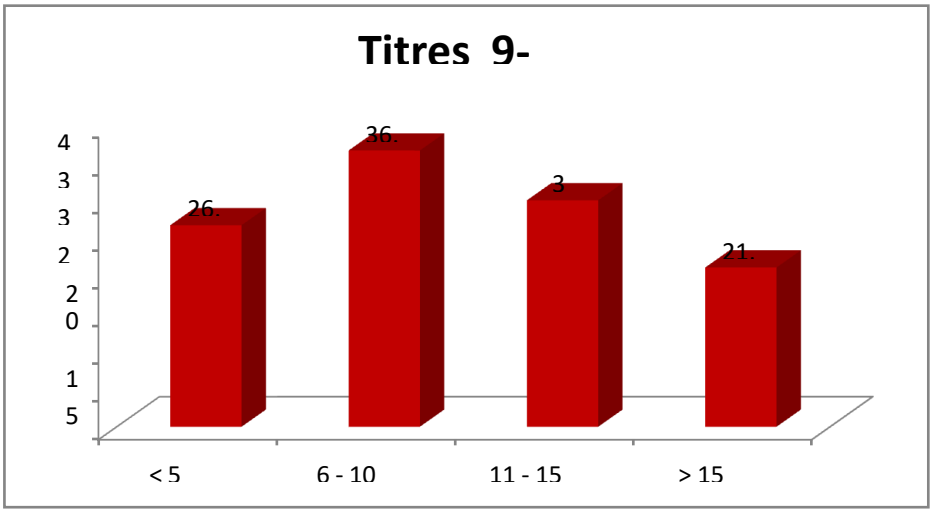

Figure No.11: Association between age group and NS1 titre levels in the study population

The association between age group and NS1 titres, In the age group of less than 5 years titre values of 9 to 11 was seen in 32(26.7%) of the children, in the age group of 6 to 10 years titre values of 9 to 11 was seen in 26(36.6%) of the children. In the age group of 11 to 15 years titre values of 9 to 11 was seen in12(30%) of the children, in age group of >15 years titre values of 9 to 11 was seen in 4 (21.1%) children.

Table No.7: Association between age group and NS1 titre levels in the studypopulation

Age Group	Titres > 11	Percentage
< 5 years	45	37.5%
6 - 10 years	22	31%
11 - 15 years	20	50%
>15 years	8	42.1%

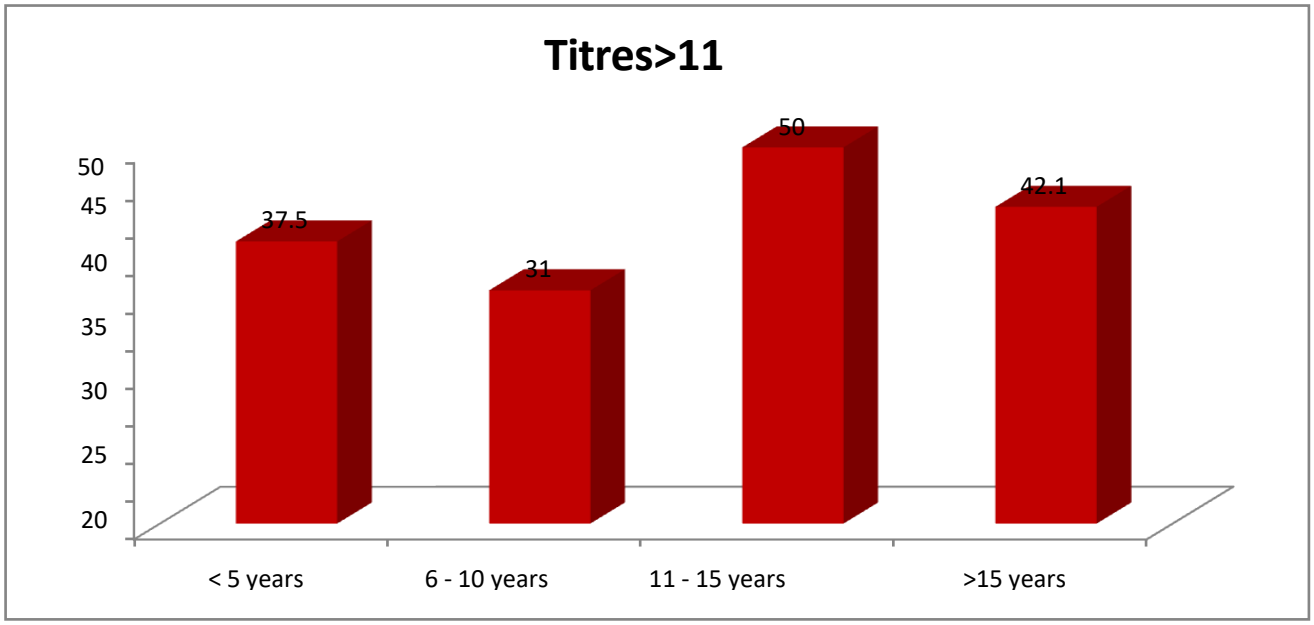

Figure No.12: Association between age group and NS1 titre levels in the study population

The association between age group and NS1 titre levels. In the age group of children <5 years titre values of > 11 was seen in 45(37.5%) children, in children with age group of 6 to 10 years titre values > 11 was seen in 22(31%) of the children. In age group of children between 11 to 15 years titre values of > 11 was seen in 20(50%) of the children. In age group of children > 15 years NS1 titres of > 11 was seen in 8(42.1%) of the children. P valuewas 0.323 which was not significant.

Table No.8: Distribution of study population according to Clinical diagnosis

Clinical Diagnosis	Number	Percentage
Mild dengue	128	51.2
Moderate dengue	97	38.8
Severe dengue	25	10
Total	**250**	**100**

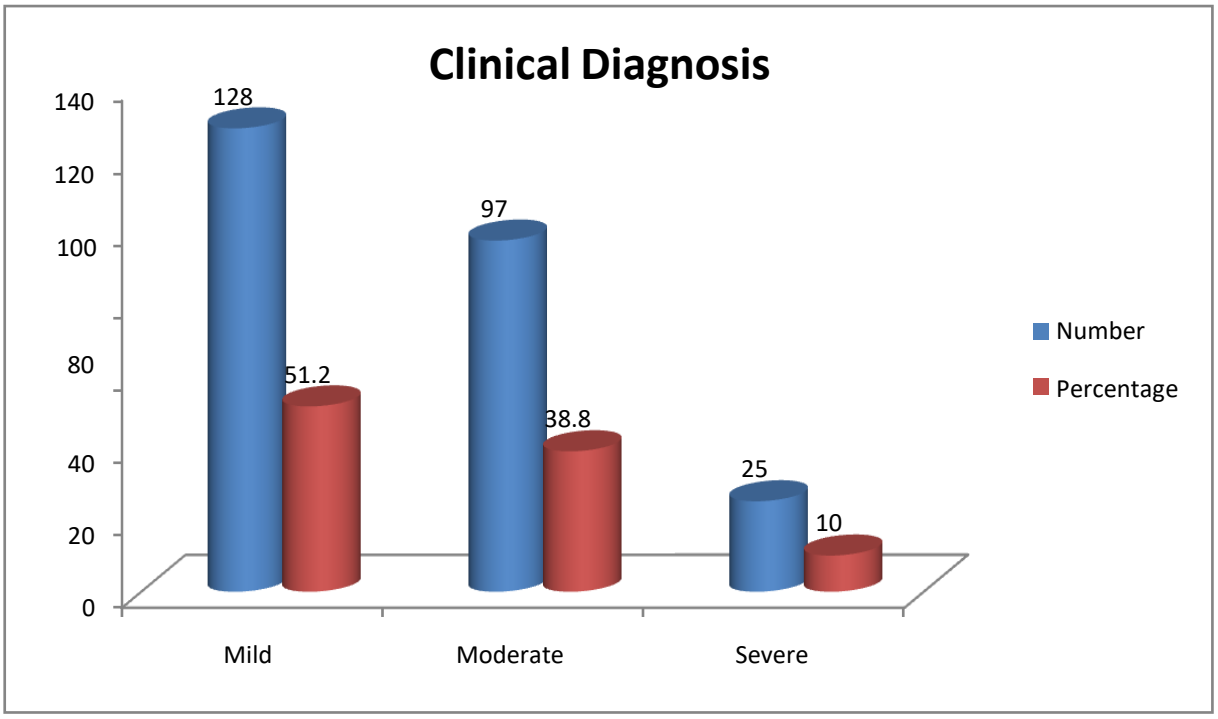

Figure No.13: Distribution of study population according to Clinical diagnosis

The distribution of study population according to clinical diagnosis. Out of 250 children Mild dengue was seen in 128(51.2%), Moderate dengue was seen in 97(38.8%) and Severe dengue was seen in 25(10%) of the children.

Table No.9: Distribution of study population according to Serology

Type of Dengue	Number	Percentage
Primary dengue	122	48.8
Secondary dengue	128	51.2
Total	**250**	**100**

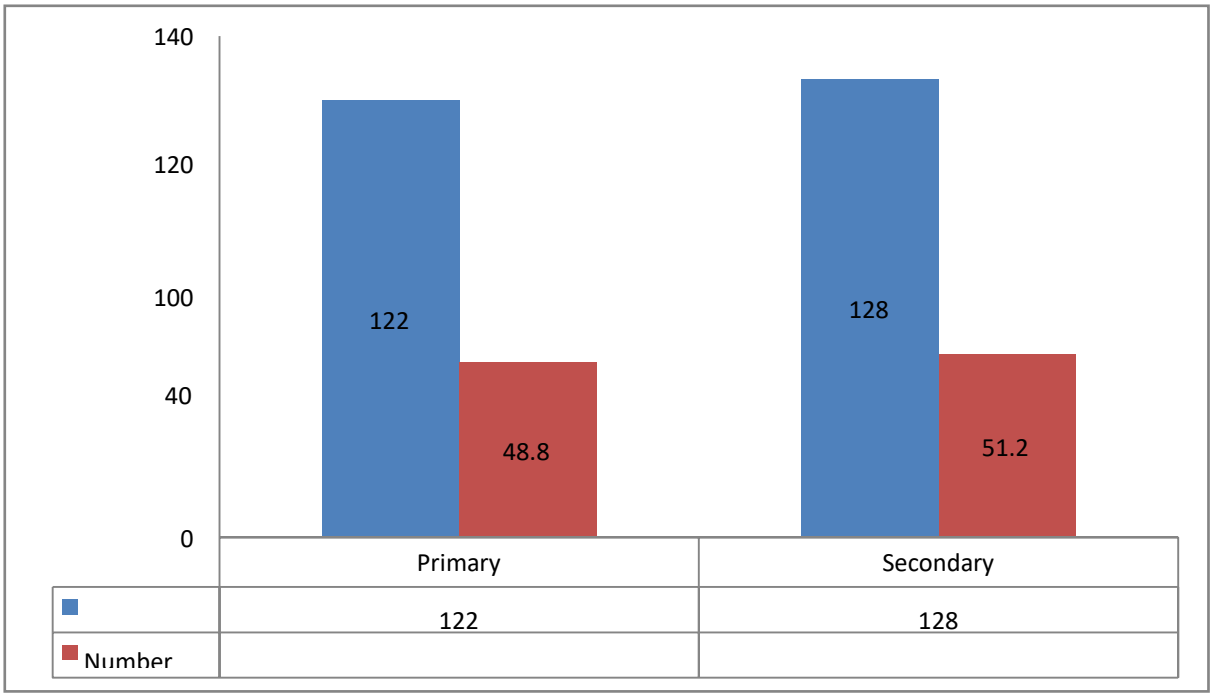

Figure No.14: Distribution of study population according to Serology

The distribution of study population according to serology. Out of 250 children 122(48.8%) had primary dengue and 128(51.2%)had secondary dengue fever.

Table No.10: Agreement between NS1 titre levels and clinical diagnosis

NS1 levels	Clinical diagnosis			P value
	Mild dengue	Moderate dengue	Severe dengue	
< 9	42(51.9)	33(40.7)	6(7.4)	0.525
9-11	43(58.1)	27(36.5)	4(5.4)	
>11	43(45.3)	37(38.9)	15(15.8)	

The Titre levels of <9 was seen in 33(40.7%) of children with moderate dengue, titre values of 9 to 11 was seen in 27(36.5%) of the children diagnosed with moderate dengue, titre values of > 11 was seen in37(38.9%) of children diagnosed with moderate dengue Titre levels of < 9 was seen in 6 children(7.4%) of children diagnosed with severe dengue, titre values of 9 to 11 was seen in 4 (5.4%) children diagnosed with severe dengue, titre values of >11 was seen in children 15 (15.8%) of children diagnosed with severe dengue. The p value was 0.525 which was not statistically significant.

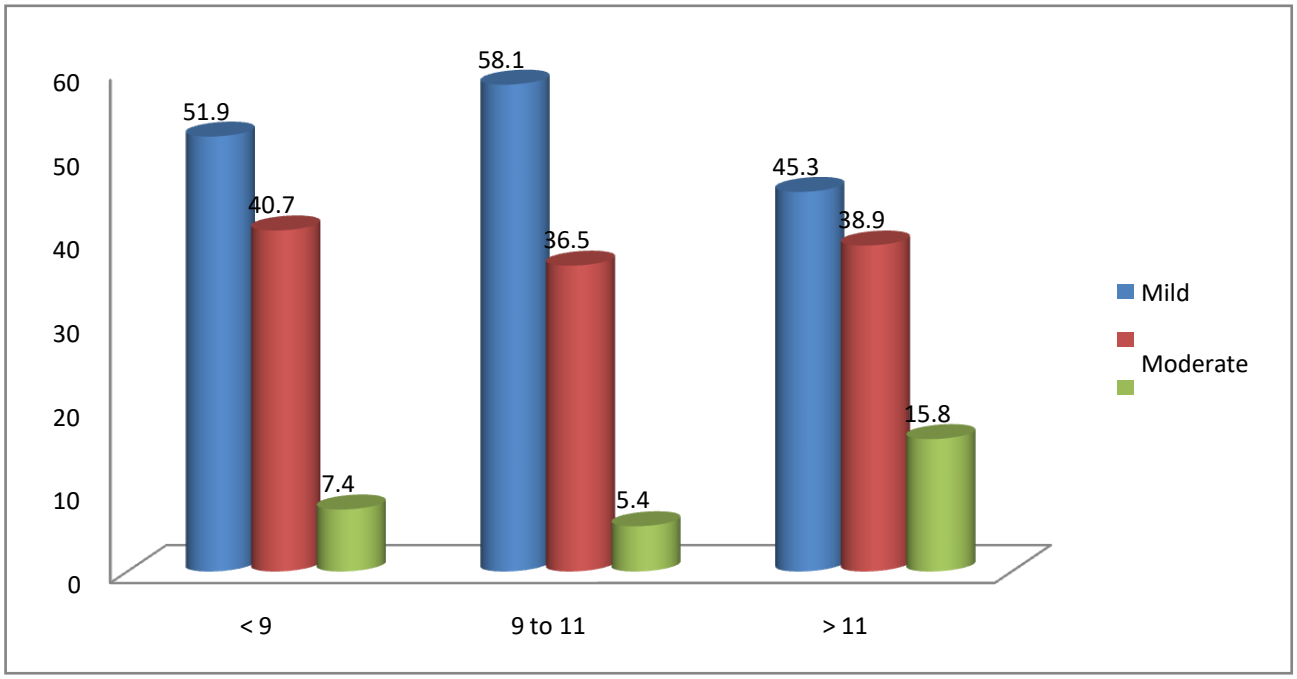

Figure No.15: Agreement between NS1 titre levels and clinical diagnosis

DISCUSSION

Demographic distribution of the studyAge

Majority of our study population (48%) were less than 5 years of age and nearly 75% of our study population was less than 10 years. The mean age of children admitted was 6.8 ± 4.93years. This was similar to Borez *et al* and Sriram Potha Pregada *et al* [59] study (the mean age group of children were respectively 6.9 years and 6.8 years.)

Sex distribution

Our study population had slightly male predominance, the male: female ratio were 1.1:1. This finding was similar to Sriram Potha Pregada *et al* [59] study.

Admission status

Most of our children were treated as inpatients (89.6%) and the remaining 10.4% were treated as out patients. This was contrary to Veasna duong *et al*[60] study where all the study population were inpatients. The children who were not admitted were mild dengue children without any warning signs.

Observation of NS1 Antigen testing and the day of illness

NS1 Antigen was found to be positive in 158(63.2%) children when donein less than 3 days of illness. NS1 antigen was positive in 92 children (36.8%) when done on 4 to 5 days of illness. Parnavitane *et al* [41] showed that NS1 Assay was found highly sensitive for dengue infection when done within day 5 of illness and similar observation was found in our study.

Severity of illness

In our study there were 51.2% of mild dengue, 38.2% of moderate dengue and only 10% were severe dengue. This can be attributed to the normal pattern of infectious diseases, where milder illnesses are more common than severe forms of the disease. Similar results were noticed by Alcon *et al* and Jeanne *et al* in their study.

Association between study population and serology

Out of 250 children included 122 (48.8%) had primary dengue and 128 (51.2%) had secondary dengue fever.

Association between age group and clinical diagnosis

When the association between age group and clinical diagnosis of mild, moderate and severe dengue was analyzed it was observed that in children less than5 years of age, Mild dengue was seen in 56.7% of the children, Moderate dengue was seen in 40% of the children and 10% of the children had severe dengue. In the age group of children from 6 to 10 years mild dengue was seen in 47.9% of the children, moderate dengue was seen in 42.3% of the children and severe dengue was seen in 9.9% of the children. In children between age group of 11 to 15 years mild dengue was seen in40% of the children, moderate dengue was seen in 47.5% of the children and severe dengue was seen in 12.5% of the children. In children more than 15 years, mild dengue was seen in 52.6% of the children, 42.1% of the children had moderate dengue and severe dengue was seen in 5.3% of the children. Statistical correlation was done which showed a p value of 0.61 which was not significant. However majority of the children were categorized as mild dengue and the common age of presentation was less than 5 years. Sriram *et al* [59] did a similar study NS1 Ag was positive in 217 (83.1%) cases and among them non-severe dengue and severe dengue was 143 cases (65.9%) and 74 cases (34.1%) respectively.

Association between age group and NS1 titre values

In the age group of less than 5 years titre values of 9 to 11 was seen in 32(26.7%) of the children, in the age group of 6 to 10 years titre values of 9 to 11 was seen in 26(36.6%) of the children. In the age group of 11 to 15 years titre values of 9 to 11 was seen in12(30%) of the children, in age group of > 15 years titre values of 9 to 11 was seen in 4(21.1%) children.

In age group of less than 5 years titre values of less than9 was seen in 43 children (35.8%) and in age group of 6 to 10 years titre values of less than 9 was seen in 23 children (32.4%). In children with age group of 11 to 15 years titre values of <9 was seen in 8 (20%) children. Children with age group of 11 to 15 years NS1 antigen titre values of <9 was seen in 7(36.8%) children. In the age group of children <5 years titre values of> 11 was seen in 45(37.5%) children, in children with age group of 6 to 10 years titre values > 11 was seen in 22(31%) of the children. In age group of children between 11 to 15 years titre values of > 11 was seen in 20(50%) of the children. In age group of children > 15 years NS1 titres of > 11 was seen in 8(42.1%) of the children. P value was 0.323 which was not statistically significant. Similar observation was seen in study conducted by Dutta *et al* study[61] where a wide range of pediatric population was included and NS1 Antigen levels with severity of dengue fever did not have any statistical significance. This study was done in teaching hospital in South India which used the similar J Mithra kit for estimation of titres similar toour study.

Association between ns1 titres and clinical diagnosis

When the association between NS1 titre levels and clinical diagnosis was correlated, Titre levels of <9 was seen in 33(40.7%) of children with moderate dengue, titre values of 9 to 11 was seen in 27(36.5%) of the children diagnosed with moderate dengue, titre values of > 11 was seen in37(38.9%) of children diagnosed with moderate dengue. Titre levels of < 9 was seen in 6 children (7.4%) of children diagnosed with severe dengue, titre values of 9 to 11 was seen in 4 (5.4%) children diagnosed with severe dengue, titre values of > 11 was seen in children 15 (15.8%) of children diagnosed with severe dengue. The p value was 0.525 which was not statistically significant.

Our study observation was similar to Study done by Kosiah *et al* [62] in Indonesia which showed that NS1 Antigen levels had a poor correlation with severity of dengue fever.

REFERENCES

1. Bhat *et al*, Threat of dengue to blood safety in dengue-endemic countries. Emerg Infect Dis 2009;15:8-11.
2. Martina BE, Koraka P, Osterhaus AD (2009) Common pathogenesis of vector borne diseases, *ClinMicrobiol Rev* 22: 564–581.
3. History of dengue, Epidemiology of dengue: past, present and future prospects Annelies Wilder-Smith
4. Evolution of dengue, Natasha Evelyn Anne Murray, 1,2 Mikkel B Quam, and Annelies Wilder-Smith
5. Wang E, Ni H, Xu R, *et al*. Evolutionary Relationship of Endemic/Epidemic and Sylvatic Dengue Viruses. *Journal of Virology* 2000: 74 (7): 3227 – 3234.
6. Journal of south east Tropical health 2011 pages 201-211, cherian *et al*. epidemics of dengue in India
7. Jounal of Tropical heath 2012, Bhatia et al evolving epidemics of dengue.
8. Martina BE, Koraka P, Osterhaus AD (2009) Dengue virus pathogenesis: an integrated view *ClinMicrobiol Rev* 22: 564–581.
9. Leurhaus 2010 *et al*, integrated approach . *ClinMicrobiol Rev* 25: 599-612.
10. WHO Global strategies of dengue epidemics 2009. Azhar EI, Hashem AM, El-Kafrawy SA, Abol-Ela S, Abd-Alla AM, Sohrab SS, *et al*. Complete genome sequencing and phylogenetic analysis of dengue
11. Joshi et al, Usmani GN, Woda BA, Newburger PE (2013) Advances in understanding the pathogenesis of HLH. Br *J Haematol* 161: 609–622.
12. Martin de Souza VA, Fernandes S, Araujo ES, Tateno AF, Oliveira OM, *et al*. (2004) Isolation of DENV3 in large Brazilian cohort. *Journal of clinical microbiology* 42: 1782– 1784.
13. Newholam, uruthus.*et al*, Advances in understanding the pathogenesis of vertical transmission of dengue, the LANCET 2009*l* 161: 609.
14. Fernandes S, Araujo ES, Tateno AF, Oliveira OM, *et al*. (2004) *Journal of clinical microbiology* 42:Koraka P, Osterhaus AD (2009) Dengue virus pathogenesis: an integrated view. *ClinMicrobiol Rev* 27:676-679
15. Henter JI, Horne A, Arico M, Egeler RM, Filipovich AH, e Diagnostic and pathogenesis of dengue,. Pediatric infectious diseases.
16. Understandingrole of non structural protein in the diagnosis of denguefever,, Pathogeni *st Pract Res Clinmicrobiology* 183–201.
17. Arico M, Egeler RM, Filipovich AH, *et al*. (2007) pathogenesis of severe dengue fever and various predictors124–131.
18. Studying the complement system in dengue Weiss G (2005) Journal ofclinical immunology rev26: 567-573.
19. Thomas L, Najioullah F, Verlaeten O, Martial J, Brichler S, Kaidomar S, *et al*. Relationship between nonstructural protein 1 detection and plasma viral load in dengue patients. Am J Trop Med Hyg. 2010;83:696-99.
20. Sekaran SD, Lan EC, Mahesawarappa KB, Appanna R, Subramanian G. Evaluation of a dengue NS1 capture ELISA assay for rapid detection of dengue. J Infect Developing Countries. 2007;1:182-88
21. Chaiyaratana W, Chuansumrit A, Atamasirikul K, Tangnararatchakit K (2008) Serum ferritin levels in children with dengue infection. *Southeast Asian J Trop Med Public Health* 39: 832–836
22. World Health Organization (2009) Dengue hemorrhagic fever: diagnosis, treatment, prevention and control (new edition). Geneva.
23. Daisy vanitha *et al*, Evaluation of the 2009 WHO dengue case classification in *an Indian pediatric cohort. Am J Trop Med*Hyg 98: 178-190
24. World Health Organization (2009) Dengue hemorrhagic fever: diagnosis, treatment, prevention and control (new edition). Geneva. /Updated WHO guidelines 2011
25. Souverine Mathews *et al* Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, Gubler DJ, Hunsperger E, Kroeger A, Margolis HS, Martinez E, et al: Dengue: a continuing global threat. Nat Rev Microbiol 2010, 8:S7–16.
26. Deaouza *et al*, Halstead SB: Dengue. Lancet 2007, Studying predictors of severe dengue 370:1644–55.
27. Mathews *et al* Experimental studies on dengue. I. Isolation, identification and modification of the virus. J Infect Dis 1952, 90:1–9. 5. Gubler DJ: Dengue and dengue hemorrhagic fever. Clin Microbiol Rev 1998, 11:480–96. PMid:9665979 PMCid:88892 6. Ross TM:4.
28. Wang E, Ni H, Xu R, Barrett AD, Watowich SJ, Gubler DJ, Weaver SC: Evolutionary relationships of endemic/epidemic and sylvatic dengue viruses.J Virol 2000, 74:3227–34.
29. Mathews Robin *et al* Dengue and dengue hemorrhagic fever. Clin MicrobiolRev 1998, 11:480–96.

30. Cornelia, Barrett AD, Watowich SJ, Gubler DJ, Weaver SC: Evolutionary relationships of endemic/epidemic and sylvatic dengue viruses. J Virol 2000,74:3227–34.
31. Kala jesi, Guzman MG, Kouri G: Dengue: an update. Lancet Infect Dis 2002, 2:33–42. http://dx.doi.org/10.1016/S1473
32. Wils Juffrie M, van der Meer GM, Hack CE, Haasnoot K, Sutaryo, Veerman AJP, *et al*. Inflammatory mediators in dengue virus infection in children: Interleukin-8 and its relationship to neutrophil degranulation. InfectImmun. 2000;68(2):702–7.Eric Halstead SB, O'Rourke EJ. Dengue viruses and mononuclear phagocytes. I. Infection enhancement by non-neutralizing antibody. J Exp Med. 1977;146:201–17.
33. Krishnamurthy, Srikiatkhachorn A, Ajariyakhajorn C, Endy TP, Kalayanarooj S, Libraty DH, Green S, *et al*. Serum interlukin levels is associated with plasma leakage in dengue hemorrhagic fever. J Virol. 2007;81(4):1592–600
34. Guzman MG, Kouri G. Dengue: an update. [2] Lancet Infect Dis. 2002;2:33-42.
35. Chakravarthi A, Kumaria R, Batra VV, Verma V. Improved detection of dengue [3]virus serotypes from serum samples-Evaluation of single-tube multiplex RT-PCR with cell culture. Dengue Bulletin. 2006;30:133-40.
36. Xu H, Pan YX, Qiu LW, Wang YD, Hao W, He LJ, *et al*. Serotype 1- specific [4] monoclonal antibody-based antigen capture immunoassay for detection of circulating nonstructural protein NS1: implications for early diagnosis and serotyping of dengue virus infections. J Clin Microbiol. 2006;44:2872-78.
37. Thomas L, Najioullah F, Verlaeten O, Martial J, Brichler S, Kaidomar S, *et al*. Relationship between nonstructural protein 1 detection and plasma viral load in dengue patients. Am J Trop Med Hyg. 2010;83:696-99
38. Sekaran SD, Lan EC, Mahesawarappa KB, Appanna R, Subramanian G. Evaluation of a dengue NS1 capture ELISA assay for rapid detection of dengue and association with thrombocytopenia J Infect Developing Countries. 2007;1:182-88.