



INCIDENCE OF NON STRUCTURAL PROTEIN (NS1) POSITIVITY & ITS CORRELATION WITH THROMBOCYTE COUNT & SERUM TRANSAMINASE LEVELS (DENGUE EPISODE-2013: A RETROSPECTIVE STUDY)

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ABSTRACT

Incidence of NS-1 antigen positivity, Platelet Count, Aspartate transaminase (AST) and Alanine transaminase (ALT) levels were retrospectively analyzed in Dengue fever, post-dengue episode-2013. NS-1 was found to be positive in 64.8% cases in present episode. Significant Serum transaminasemia (raised AST and ALT levels) & thrombocytopenia were found in both NS1 + ve as well as NS-1 negative groups. Aspartate transaminase (AST) increase was more marked than alanine transaminase(ALT). Both AST & ALT showed linear increase and inverse correlation with the degree of thrombocytopenia in NS1+ve group ; however, linear correlation was lacking in the NS-1 negative group. Relatively low incidence of NS-1 positivity is indicative of high probability of dengue re-infection. Marked thrombocytopenia associated with significantly raised serum transaminase level in NS-1 negative group corroborates the possibility of secondary infection. Thus negative NS-1 antigen test report does not necessarily exclude the diagnosis of Dengue viral infection. It is recommended that Positive NS1, significant thrombocytopenia and moderately elevated AST levels within 3 – 4 days of onset of disease, may be conjointly used as distinct dengue morbidity triad for distinguishing morbid from benign patients. The criteria would help identify the patients needing hospitalization and intensified cares. However NS1 may not be relevant in endemic zones, migrants, and frequent travellers with high probability for re-infection.

KEY WORDS & ABBREVIATIONS : Dengue Nonstructural Protein1 (NS1), Dengue morbidity criteria Thrombocytopenia(TCP), Platelet count (PC) Transaminasemia (Raised AST /ALT Level in serum).



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INTRODUCTION

Dengue is a common mosquito-borne viral infection. Its incidence has attained unprecedented proportions in recent times.^{1, 2}, affecting up to 100 million population, each year, across the tropical world. Four Dengue serotypes (DENV-1-4) have been reported and all of these serotypes have been detected in the previous dengue outbreaks³. The infection provides life-long immunity against the specific serotype. Thus the patients are vulnerable to re-infection with other serotypes. The virus genome codes for three structural proteins and seven non structural proteins³. The non structural protein (NS1 antigen) is replicated during viral replication in the host cell^{4,5} and has been shown to become positive on the very first day of viral infection⁶. It has thus been introduced as the earliest diagnostic marker for dengue infection. The sensitivity of NS-1 detection in Dengue infection has been reported to vary from 70% to 100 %, in case of primary infection. However, the sensitivity has been reported to be considerably reduced in endemic zones with high probability for re-infection with other serotypes.⁷ NS-1 negativity thus does not necessarily exclude dengue in such cases. NS-1 therefore needs to be suitably supplemented with additional markers so as to delineate morbid dengue cases. We have, therefore, retrospectively analyzed the incidence of NS1 Positivity, degree of thrombocytopenia (a characteristic feature of dengue infection) and serum transaminase

(AST & ALT) levels during dengue outbreak 2013 so as to evaluate- a). Incidence of NS1 positivity b). Degree of thrombocytopenia and transaminasemia c). Whether the triad of NS1, PC, and AST /ALT can be used as a definitive criteria to distinguish dengue morbid and benign patients.

MATERIALS AND METHODS

This retrospective study was carried out by the retrieval of patient data of dengue cases available on Hospital information system of HAHC hospital, Jamia Hamdard during the year 2013. Total of 117 clinically suspected cases of dengue with history of fever 1-4 days prior to reporting in the hospital, were analyzed. Patients having other associated hepatic disorders and / or frank Jaundice were excluded. 23 of the dengue suspected cases did not show any laboratory evidence (NS1-negative, PC & Transaminase levels within normal limits) were therefore excluded from further analysis. The number of cases analyzed was thus restricted to 94 evidence based dengue cases - Male -52 (55.4%) & Female 42 (44.6 %); age 15-75yrs). Dengue NS1 antigen test was carried out by rapid antigen detection kit. Platelet count and Transaminase assays were carried out on automated equipments (cell counter/fully automated biochemistry analyzer)

RESULTS

Table 1
Incidence of NS-1 positive and negative cases (%)

	N	%	NS-1 +VE	NS-1 -VE
Male	52	55.4	35	17
Females	42	44.6	26	16
TOTAL	94		61 64.8%	33 35.2%

Table 2
SERUM. TRANSAMINASE (AST AND ALT) LEVELS IN DENGUE CASES. –(Over all data)

TOTAL(n=94)	AST (IU/l)	ALT (IU/l)
MEAN , S.D & Range	131.9 ±152.8 (20 – 1019)	105 ±116.2 (18 – 739)

Table 3
S. AST & ALT STATUS IN NS1 + VE AND – VE GROUPS:- DEGREE OF ELEVATION

AST LEVELS (IU/l)				
AST ELEVATION	NS1+ve		NS1-ve	
	N	%	N	%
A. NORMAL	11	18%	8	24.2
B. MILD (2.5 FOLD INCREASE)	23	37.7%	16	48.4
C. MODERATE (5 FOLD INCREASE)	16	26.3%	5	15.2
D. MARKED (>5 FOLD NCREASE)	11	18%	4	12.2
TOTAL NO/% CASES WITH ELEVATION	50	82%	25	75.8% (76%)
ALT levels (IU/l)				
ALT ELEVATION	NS1+ve		NS1-ve	
	n	%	n	%
A. NORMAL	5	8.2%	3	9
B. MILD (2.5 FOLD INCREASE)	38	62.3%	21	64
C. MODERATE (5 FOLD INCREASE)	13	23.3%	6	18
D. MARKED (>5 FOLD NCREASE)	5	8.2%	3	9
TOTAL NO/% CASES WITH ELEVATION	56	91.8%	30	91%

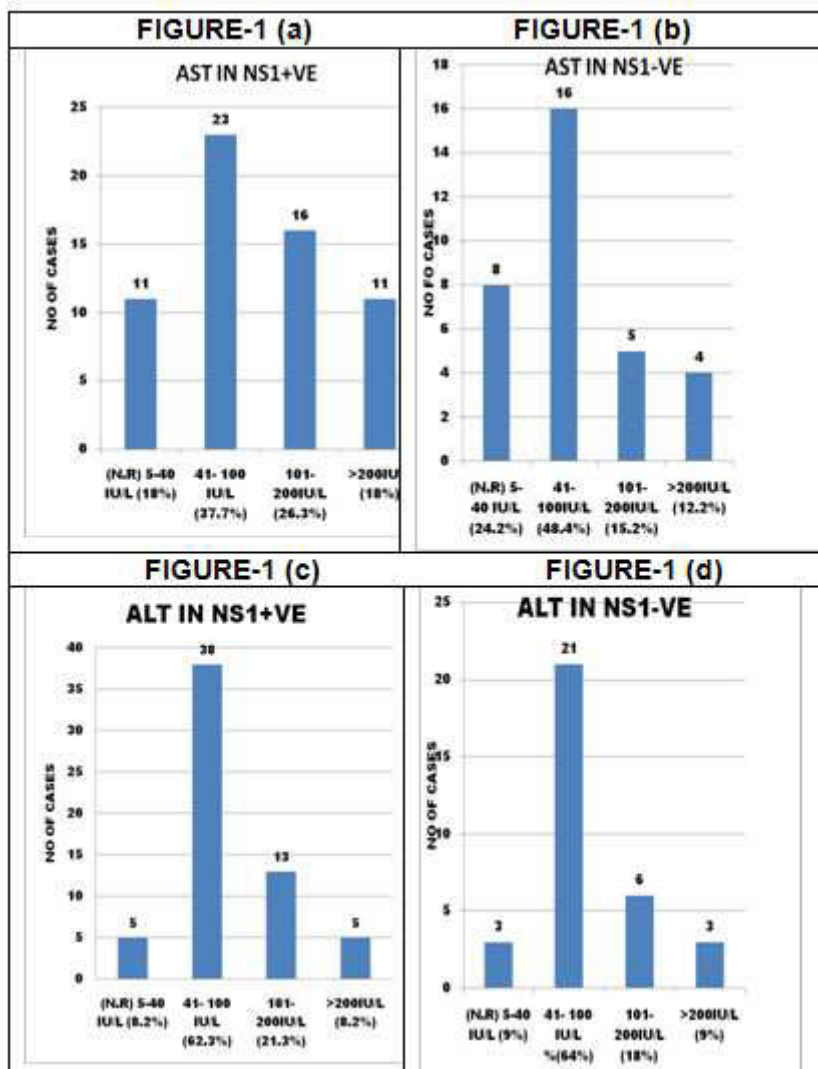
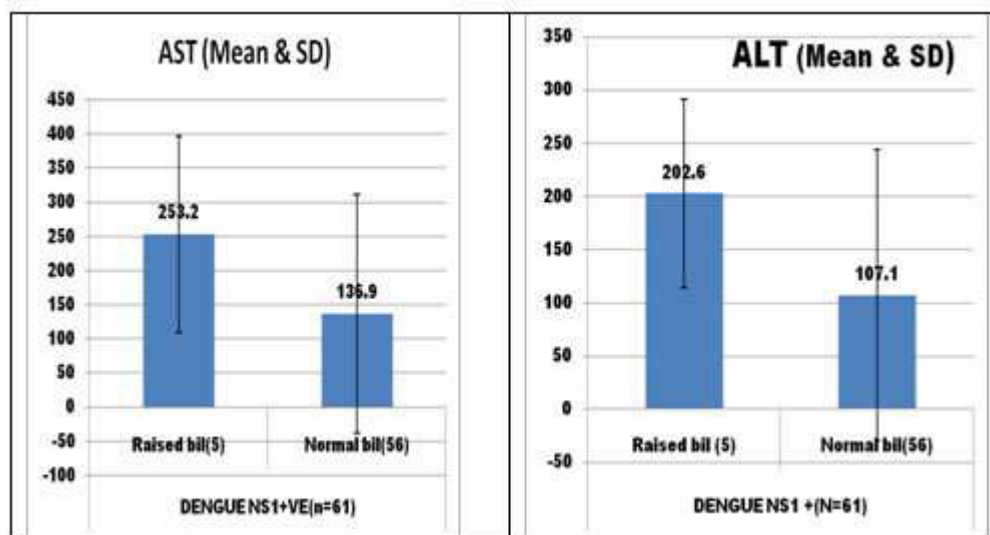


Table 4
AST and ALT levels in relation to Bilirubin level in NS1 +ve cases

	DENGUE NS1+VE(n=61)			
	Raised Bil (n=5) S. Bil.- 1.2 – 1.8 mg/dl		Normal Bil (n=56) S. Bil.- < 1.2 mg/dl	
	AST	ALT	AST	ALT
MEAN/ SD	253.2 ±143.2	202.6 ±88.3	136.9 ±174.2	107.1 ±136

Figure 2



PLATELET COUNT

Table 5
NS1 +VE GROUP :- DISTRIBUTION OF CASES ACCORDING TO PLATELET COUNT (NS-1 + and - GR)

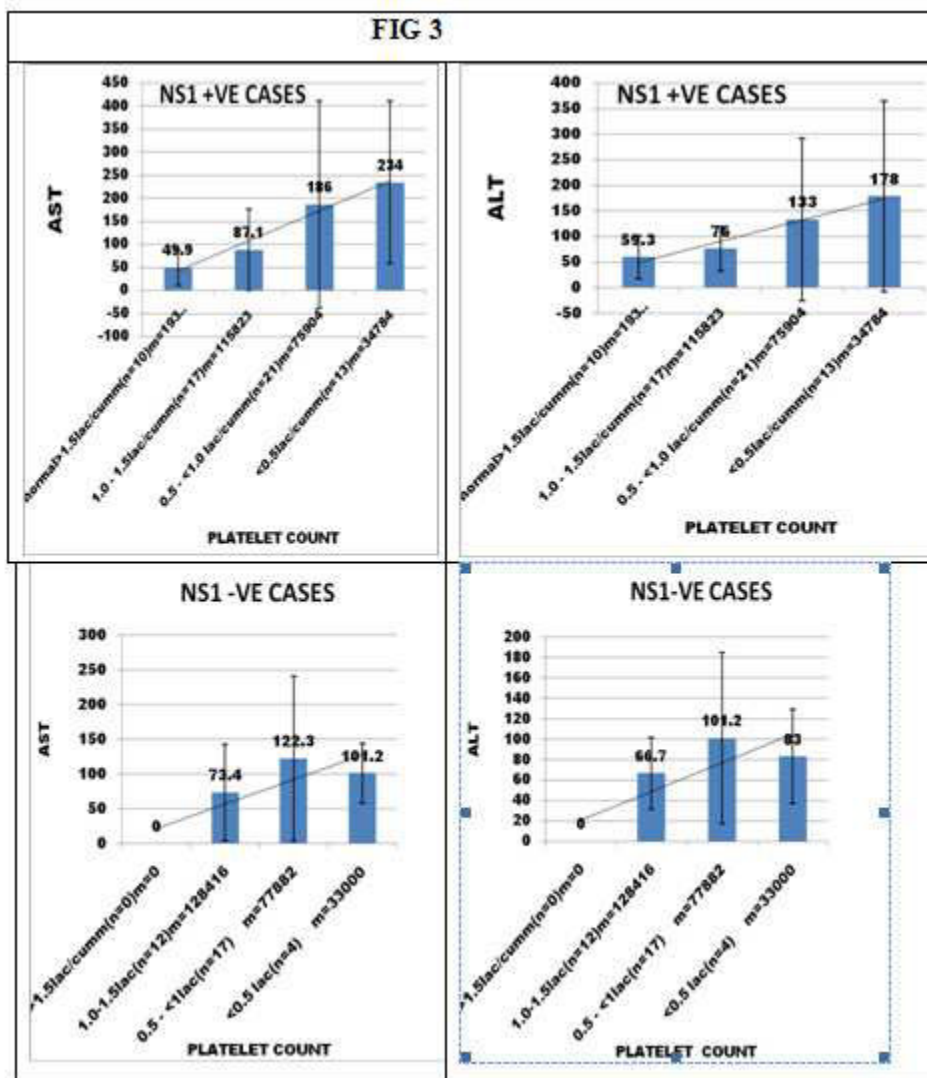
DEGREE OF THROMBOCYTOPENIA (TCP)	NS1+ve			
	n	%	Mean (lac/cumm)	SD
Gr A. NORMAL PC >1.5lac/cumm	10	16.3	1.9	0.44
Gr B. MILD TCP 1.0 -1.5lac/cumm	17	27.8	1.1	0.15
Gr C. MODERATE TCP 0.5 - <1.0 c/cumm	21	34.5	0.75	0.10
Gr D. MARKED TCP <0.5lac/cumm	13	21.4	0.34	0.10
TOTAL NO/% CASES WITH TCP	51	83.7%		

DEGREE OF THROMBOCYTOPENIA (TCP)	NS1-ve			
	Number	%	Mean	SD
Gr A. NORMAL PC >1.5lac/cumm	Nil	-	-	-
Gr B. MILD TCP 1.0 -1.5lac/cumm	12	36.3	1.28	0.15
Gr C. MODERATE TCP 0.5 - <1.0 c/cumm	17	51.5	0.77	0.11
Gr D. MARKED TCP <0.5lac/cumm	4	12.2	0.33	0.12
TOTAL NO/% CASES WITH TCP	33	100%		

Table 6
ELEVATION(FOLD INCREASE ,F.I.) IN AST/ALT LEVELS IN RELATION TO DEGREE OF THROMBOCYTOPENIA [NS1+VE & - VE GROUPS] (CORRELATION)

NS1 Positive Group						
THROMBOCYTOPENIA (TCP) With Mean Platelet count (PC)	AST				ALT	
	N	%	Mean & SD	F.I.	Mean & SD	F.I.
Gr.A. NORMAL M= 1.93 lac/ c.mm	10	16.4	49.9 ±39.5	1.25	59.3 ±42.3	1.48
Gr B. MILD TCP M=1.15 lac/ c.mm	17	27.8	87.1 ±88.1	2.17	76 ±44.8	1.9
Gr.C.MOD TCP M=0.759lac/ c.mm	21	34.5	186 ± 225.1	4.6	133 ± 159.1	3.3
Gr D. MARKED M=0.347lac/ c.mm	13	21.3	234 ±177.1	5.8	178 ± 186.9	4.4
Correlation Coefficient (r)	AST/PC 0.0047			ALT/PC 0.03		

NS1 Negative Group						
THROMBOCYTOPENIA (TCP) With Mean Platelet count (PC)	AST				ALT	
	N	%	Mean & SD	F.I.	Mean & SD	F.I.
Gr.A. NORMAL 1.93 lac/ c.mm	-	-	-	-	-	-
Gr B.MILD TCP 1.15 lac/ c.mm	12	36.3	73 ± 70	1.82	66.7 ±35.4	1.6
Gr C.MOD. TCP 0.759 lac/ c.mm	17	51.5	122 ± 119.3	3.05	101.2 ±83.9	2.52
Gr D. MARKEDTCP 0.347 lac/ c.mm	4	12.2	101.2 ±42.8	2.5	83 ±46	2.07
Correlation Coefficient (r)	AST/PC 0.18			ALT/PC 0.20		



DISCUSSION

As per WHO 2009 classification Dengue has been classified into a). Probable (Benign) Dengue cases b).Critical Dengue (Dengue hemorrhagic fever, dengue shock syndrome). In the early phase, the diagnosis is primarily based on the demonstration of NS-1 antigen; severe thrombocytopenia being the only definitive lab parameter used as a warning sign. However, in secondary dengue cases, with high probability of negative NS-1 test, role of other key markers (PC & Transaminase level), need to be evaluated along with NS-1 test for diagnosis & identification of morbid dengue cases.

Incidence of NS1 Positivity

Positivity of NS-1 antigen test has been reported to vary considerably, depending upon –a).The stage of testing (the test being positive from day-1 to day 4 only) b) First (Primary) dengue infection c). Dengue re-infection by a different serotype (common in endemic zones & migrants with a high probability of secondary re-infection). Different workers have reported variable NS1 positivity. Singh MP et al (2011)⁸ reported NS1 to be positive in 71-100% of cases till day 3 of fever. Fauziah Md Kassim et al (2011)⁹ reported the diagnostic sensitivity of NS-1, PCR, IgM, & IgG as 32.2 % , 38.5 % , 40.9% and 36.1% respectively. Kumarasamy et al,

(2006)⁶ reported the sensitivity of NS1 antigen in secondary dengue to be reduced from 97% (Primary infection) to 70%. Dussart et al (2006)⁷ reported the sensitivity of NS1 test to vary from 88.7%-100% , according to the stage of illness (only 45% beyond seventh day). Lapphara et al (2008)¹⁰ reported much lower NS-1 positivity(63.2% only) , explained on the basis of higher prospects of acute secondary dengue cases

NS-1 positivity during 2013 outbreak

In the present outbreak NS-1 positivity was found to be 64.8% only, (Table-1) indicating a high probability of secondary dengue infection (endemic nature of the study zone having frequent annual dengue outbreaks). However, delay in NS1 testing (beyond 4 days of onset of disease) may also account for NS negative results. It is further implied that NS-1 negative test does not necessarily exclude the possibility of dengue infection specially in the first 3-4 days of onset of the symptoms/disease. Other associated markers like Transaminase level & Thrombocyte count need to be taken into account individually & jointly, while evaluating such NS-1 negative cases.

Serum Transaminase (AST & ALT) Levels (Table- 2, 3, 4 & Fig 1)

In the present study Mean AST and ALT level was found to be 131.9 ± 152.8 & 105 ± 106.2 (Table- 2). AST level was found to be increased in 82 % in NS1 Positive group and 76 % cases in NS1negative group ALT levels were found elevated in > 90 % cases in both NS1 positive and negative groups. AST & ALT levels showed marked elevation in 18% & 8.2% in NS-1 (+) gr; respective values for NS-1 (-) gr were 12.2% & 9 % cases (Table- 3 & Fig 1). Different workers have reported mild to moderate increase in serum transaminase (AST & ALT) levels^{11,12,13,14}. Manzhi wong¹² reported 1-10 fold AST elevation. 63% cases showed mild elevation (1-5 fold) , 27.5% cases showed moderate (5-10 fold) and 10% showed marked elevation (> 10 fold) increase . In the present study the fold increase in AST/ALT levels was much higher in NS1+ve

group compared to NS1-ve group. 8% cases in NS1 positive group were found to have mild elevation in bilirubin level (1.0-1.8mg/dl,) with AST and ALT values being approx 1.8 fold elevated; (Table-4 & Fig 2) In the present study AST showed higher fold increase (1.8 – 5.8 fold) while ALT showed 1.7-4.4 fold increase) in NS1 +ve group compared to NS1-ve group. AST elevation has been reported to be quicker, more marked & reverting back to normal ,sooner than ALT.¹¹ Elevation in AST/ALT and NS1 antigen is proportional to the degree of virulence. Higher rise in AST has been attributed to some non hepatic source (monocytes).¹⁵ It has been suggested that NS1 is endocytosed by hepatocytes and its accumulation depends on the virulence.¹⁶ Kulkarni et al¹³ concluded that the inclusion of NS1 significantly increases the dengue detection rate .Further, thrombocytopenia was more consistent in dengue positive rather than dengue negative subjects.

Platelet Count

Incidence of thrombocytopenia. (Table-5).

NS1+ve group- 84% of the cases showed thrombocytopenia However 16.3% cases did not show any thrombocytopenia .In the NS1-ve group- all the cases (100%) showed thrombocytopenia

Degree of thrombocytopenia (Table-5).

NS-1 positive & negative groups showed mild to moderate thrombocytopenia in 64% & 77 % cases & marked thrombocytopenia (PC<0.5lac) in 21.4%r and 12.2% respectively. Higher incidence of thrombocytopenia was observed in NS-1 negative cases; this corroborates with the possibility of dengue re- infection

S.Transaminase & Thrombocyte Count in NS1 Positive & NS-1 Negative Groups : Correlation.(Tab 6 & Fig 3)

NS1+ve group- Both AST & ALT showed linear but inverse correlation with the degree of thrombocytopenia. In this category AST / ALT levels showed 1.2 to 5.8 fold increase. The linear inverse correlation was lacking in NS1 - Ve group- However the fold increase in AST and ALT levels were much lower (1.6 to 2.5

fold), compared to NS-1 positive group. The fold increase in AST was more marked, compared to ALT in both NS 1 + ve and NS1 – ve group. Thus NS-1 positive cases with moderate to severe thrombocytopenia & elevated AST level may be used as criteria for identifying & differentiating benign dengue patients from Dengue morbid patients. Earlier studies have reported mild to moderate increase in serum AST/ALT levels.^{11,12}

CONCLUSION

Negative NS1 antigen in the first 3-4 days of illness does not exclude the diagnosis of Dengue infection, especially in endemic zones. The triad of laboratory parameters comprising of NS1 antigen, Platelet count and serum AST levels may be used as Dengue morbidity criteria for distinguishing domiciliary and hospital based care.

Conflict of interest - Conflict of interest -Declared none.

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