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A PROSPECTIVE STUDY OF PREDICTORS OF MORTALITY IN ACUTE RESPIRATORY FAILURE IN H1N1 INFLUENZA.

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ABSTRACT

Aim: H1N1 pneumonia with respiratory failure is associated with significant mortality and morbidity. There is a need to evaluate independent predictors of mortality. **Methodology:** Patients with suspected H1N1 pneumonia presenting to emergency at JSS Hospital, Mysuru during February-June 2015 were screened for H1N1. Patients with H1N1 were evaluated for independent predictors of mortality. Survivors were followed up at 3rd month and 1 year for long-term sequelae and activity limitation. **Results:** 175 patients were screened for H1N1 and 71 were confirmed, of which 52 needed ICU admission and 24 needed mechanical ventilation. Nine subjects were given a trial of NIV before mechanical ventilation. Twenty eight subjects could be managed with NIV alone. A very high mortality 24/71 (33.8%) was observed inspite of anti-viral treatment. Cox regression analysis identified APACHE II score (>10), comorbidities and rise in platelet counts (<15% from baseline) were independently associated with mortality. No long term sequelae or activity limitation was observed in survivors. **Conclusion:** H1N1 pneumonia with respiratory failure is associated with very high mortality. Each of the above 3 variables APACHE II score (> 10), comorbidities and rise in platelet counts (<15%from baseline) were associated with a greater than 3 times the hazard for mortality.

KEYWORDS: APACHE II, H1N1, Influenza, long-term sequelae, mortality.

1. INTRODUCTION

The first influenza pandemic in 1918, Spanish influenza, affected 500 million worldwide leaving no inhabited regions untouched and killed 40-50 million people. In India around 10-20 million people were affected with 10 percent mortality.[1] The most recent pandemic (novel H1N1 influenza) in 2009 during April to August involved 168 countries[2] with 277,607 cases and 3205 deaths.[3] WHO declared H1N1 a public health emergency in April 2009 and a global pandemic in June 2009[2], which lasted till August 2010. In the post pandemic phase in 2010, the same virus continued as seasonal virus with sporadic or localized outbreaks, especially in India and New Zealand.[4]

H1N1 reappeared in 2012-2013 in northern and western states of India during winter. The resurgence since December 2014 was worse with 33000 confirmed cases, more than 2000 deaths[5], and increased to 2990 deaths in 2015. In 2014, genetic sequencing of H1N1 virus in India showed mutations with enhanced virulence.[6] H1N1 continues as an epidemic in many countries including India with high mortality among patients needing hospital admission. WHO is continuing surveillance on H1N1 and the strain is one of the 3 strains recommended for influenza vaccine in 2017.[7]

H1N1 pneumonia has high mortality necessitating early diagnosis and effective intervention to reduce the health care burden. Various factors such as younger age[8–10], early initiation of oseltamivir[9], hypoxia[11,12], altered mental state[12], pregnancy[13,14], comorbidities like heart disease[8], diabetes, renal failure needing dialysis[10], obesity[15], lymphopenia[11], thrombocytopenia[16], elevated LDH[8], elevated creatine kinase[9] need for invasive ventilation(8), APACHE II scores[17,18] were observed as independent predictors of mortality. Anecdotal reports suggested that H1N1 survivors developed pulmonary fibrosis.[19] This study was conducted to identify independent predictors of mortality in H1N1 patients needing hospital admission and to identify any long-term clinical, radiological and functional sequelae at 3 months and 1 year.

2. MATERIALS AND METHODS

2.1 Study design: We carried out a prospective observational study by evaluating all patients with acute onset of fever, cough and breathlessness with respiratory failure, infiltrates in chest x-ray attending emergency department (ED) at 1800 bedded tertiary care university teaching hospital, Mysuru, between February–June 2015. Flow of patients and subsequent management has been depicted in Fig 1. Patients requiring mechanical

ventilation, requiring a fraction of inspired oxygen (FiO2) greater than or equal to 60%, receiving intravenous infusion of inotropes or vasopressors were admitted to ICU and rest were managed in emergency ward. A trial of non-invasive ventilation (NIV) was considered as per the recent guidelines for acute respiratory failure.[20] All patients were screened for H1N1 by throat swab on the day of admission. One sample of nasopharyngeal swab in non-mechanically ventilated patients was taken under strict aseptic precautions from the posterior pharyngeal wall by single circumferential swipe and bronchial-aspirate samples were obtained from patients on mechanical ventilator and put in sterile container containing 3ml of viral transport media –HiViral (Himedia) and sent in cold chain, to Kasturba Medical College, Manipal, a government approved lab for detecting H1N1. Samples reached the lab within 24-48 hours. The samples were analyzed by Taqman Real-Time PCR (CDC protocol) for influenza-A, influenza-B, swine flu-A, swineflu-H1 in accordance with published guidelines from the U.S. Centers for Disease Control and Prevention (CDC). Results were obtained on 5th day from the day of sample dispatch. Patients with confirmed H1N1 were included in this study. Oseltamivir was administered on the first day of admission.

2.2 Data collection: Demographic data, details of influenza contact, symptoms, number of days between onset of symptoms and start of oseltamivir, comorbid conditions, alcohol and tobacco consumption and clinical measurements such as BMI, pulse rate, blood pressure, respiratory rate and oxygen saturation were recorded. Chest X ray, ECG, 2D Echocardiography, Arterial Blood gas analysis, Complete hemogram, serum electrolytes and liver and renal function tests and repeat platelet count on 3rd day were also recorded. Severity of illness was assessed using Acute Physiology and Chronic Health Evaluation II (APACHE II)[21] at admission. Outcome variables included length of hospital stay (includes stay in ICU and wards) and mortality. The threshold platelet count used to define thrombocytopenia was 150×10^9/l or below, based on local laboratory protocols and current literature.[22] Obesity was defined as a BMI >30.[23] Presence of co-morbidities and chronic conditions like asthma, chronic obstructive pulmonary diseases (COPD), congestive heart failure, neuromuscular disorders, diabetes mellitus, hypertension, obesity, coronary artery diseases, immunosuppressive status or renal failure were recorded. Arterial Blood gas analysis was done in ABL FLEX 800 machine (Radiometer), Serum electrolytes in DIESTRO electrolyte analyzer, liver and renal function tests in TOSHIBA TBA 120 FR a fully automated chemistry analyzer. Complete hemogram was analyzed in NIHON KOHDEN (5 part differential cell counter) and

SYSMEX (6 part differential cell counter). Hemoglobin was quantified by Cyanmeth hemoglobin method and platelet count and total count was quantified using Flow-cytometer. Spirometry was performed according to ATS standards after 3 months and one year after discharge from the hospital.

The Institutional Ethics Committee approved the study with IEC number JSSMC/IEC/04/6756/2014-15. Informed consent from patient/legal representative was taken prior to inclusion in the study.

2.3 Analysis: Descriptive data are presented as frequencies (percentages) for discrete variables and as means (SDs) or medians (interquartile ranges [IQRs]) for continuous variables. For comparisons between two groups, Mann-Whitney U test was used or, when appropriate, the two-sample *t*-test. χ^2-test was used to evaluate categorical factors. Cox regression univariate and multinomial analysis and Kaplan Meier was used for survival analysis. Receiver operating characteristic (ROC) curve were constructed for APACHE II score, rise in platelets 3 days after treatment with oseltamivir and the cut-off value with highest sensitivity and specificity was selected as threshold. All statistical tests were 2-tailed, and factors were considered statistically significant at p <0.05. IBM SPSS version 22 and CDC Epi Info version 7 was used for analysis.

3. RESULTS
We screened 175 patients presenting to ED for H1N1 and 71 (25 men, 46 women) were confirmed to have H1N1. Symptoms at presentation were cough (98.6%), dyspnea (97.2%), fever (81.7%), sore throat (43.6%), head ache (16.9%), hemoptysis (7.04%), vomiting (9.8%) and loose stools (2.8%). Comorbidities were observed in 49.2% of patients and type2 diabetes mellitus (24%) was the most common. All patients had bilateral radiological opacities (33 alveolar, 32 interstitial, 5 both alveolar and interstitial) except one with a unilateral opacity. The mean duration of onset of symptoms to presentation was 5.30±2.73 days. Mean duration of dyspnea before hospitalization was 2.6±2.11 days. Mean time from hospitalization to ICU admission was 1±0.61 day. Mean duration of symptoms to initiation of oseltamivir was 5.56±2.69 days. Mean APACHE II score at admission was 7.64±3.77. Fifty-two patients (73.2%) needed ICU and 16 patients were managed in emergency ward. Three succumbed within 1 hour of admission. In the ICU, 24 patients needed invasive mechanical ventilation. Fifteen patients required immediate invasive ventilator support and 9 patients were given a trial of non-invasive ventilation before invasive ventilation. The other 28 patients on non-invasive ventilation recovered without needing mechanical ventilation.
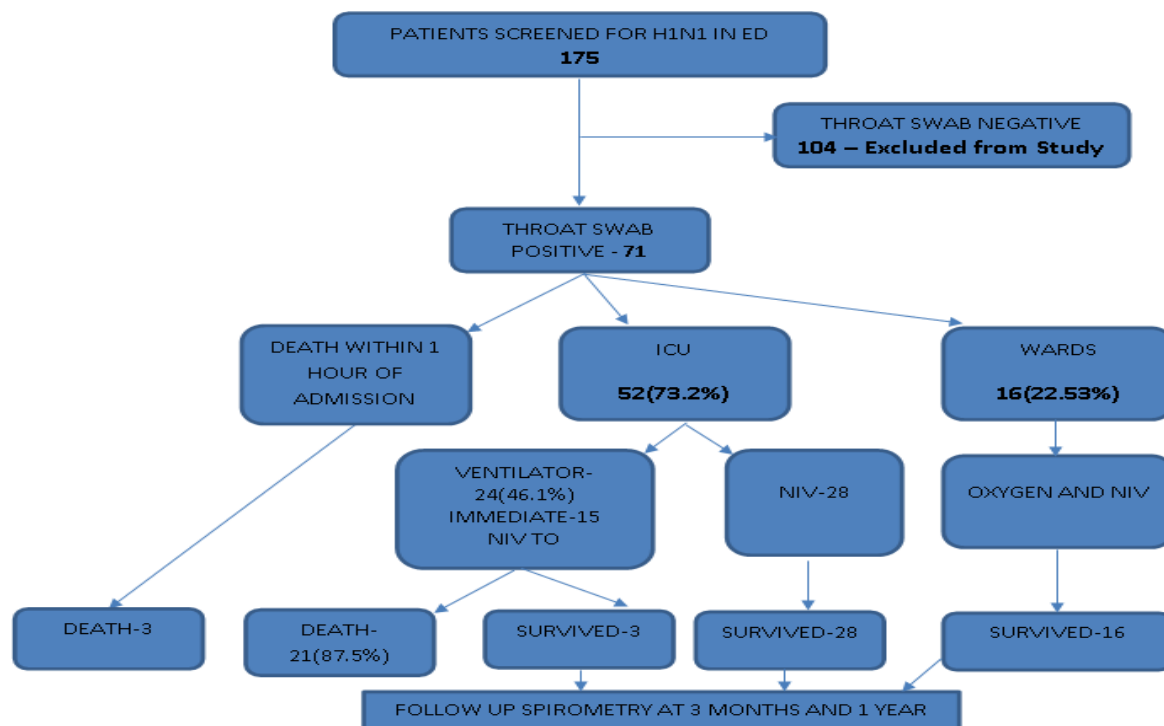
Fig. 1 Flow chart depicting number and proportions of patients screened, included in the study, needing ICU care, mechanical ventilation, NIV, survivors and deaths.

Two patients with acute kidney injury responded to medical management. Nosocomial infections were noted in 12 patients of which ventilator associated pneumonia (after more than 4 days of ventilation) was seen in 10 patients (14.1%), catheter related blood stream infection (CRBSI) in 2 patients (2.82%). Organisms isolated from endotracheal aspirate from VAP patients were Actinetobacter (n=8), Pseudomonas (n=2). Univariate analysis of factors associated with mortality are depicted in Table 1.

Table 1 Clinical profile and Univariate cox proportional hazard analysis of factors influencing mortality in H1N1 pneumonia patients.

aSr.No	Characteristics	Total n=71	Survivors n=47	Non survivors n=24	p value
1	Age, mean(SD)	44.42±12.81	44.19±14	44.87±12	0.833
2	Female sex, n (%)	46(64.78)	32(68.08)	14(58.3)	0.581
3	Comorbidities, n (%)	35(49.29)	19(40.4)	16(66.6)	**0.036**
4	Obesity n (%)	3(4.20)	2(8.33)	1(2.13)	0.262
5	Diabetes (type2) n (%)	17(24)	7(14.89)	16(66.6)	**0.0130**
6	Hypertension, n (%)	9(12.68)	4(16.67)	5(10.64)	0.475
7	COPD n (%)	6(8.45)	4(16.67)	2(4.26)	0.184
8	Asthma n (%)	7(9.80)	2(8.33)	5(10.64)	0.559
9	HIV n (%)	2(2.82)	1(4.17)	1(2.16)	1.00
10	IHD n (%)	2(2.82)	1(4.17)	1(2.13)	1.00
11	Smoker n (%)	16(22.5)	8(17)	8(33.3)	0.12
12	Thrombocytopenia at admission n (%)	27(38)	14(29.8)	13(54.2)	**0.045**
13	Time course of illness (days) mean(SD)				
14	Symptoms to admission	5.30(2.73)	5.25(2.74)	5.41(2.76)	0.910
15	Dyspnea to admission	2.67(2.11)	2.34(1.56)	3.33(2.83)	0.789
16	Hospitalization to ICU	1.00(0.68)	1.20(0.81)	1.04(0.2)	0.411
17	Symptoms to start of oseltamivir	5.56(2.69)	5.53(2.70)	5.62(2.74)	0.89
18	APACHE II score at admission, mean (SD)	7.64(3.82)	6.25(2.93)	10.37(3.78)	**0.000**
19	PaO2/FiO2,mm Hg (at admission), mean (SD)	206(105.01)	252.68(95.30)	114.96(47.9)	**0.0001**
20	Hypotension at admission,n (%)	19(26.76)	8(17)	11(45)	**0.009**
21	Initial MAP, mean(SD)	87.71(11.01)	89.5(8.6)	84.1(14)	0.060
22	Vasopressor usage(%) during course of stay	15(21.12)	2(4.3)	13(54.2)	**0.001**
23	NIPPV during course of stay, n (%)	37(52.11)	24(51.1)	13(54.2)	0.80

Median length of hospital stay was 7(IQR 4-18) days. We observed high ICU mortality of 40.38% and hospital mortality of 33.80%. Only 3 of 24 patients who needed mechanical ventilation and all who needed only non-invasive ventilation survived. There were no deaths in patients shifted to ward after observation at the ED. The causes of death were ARDS (22 patients), VAP (2 patients).

Comparison of survivors and non-survivors: Patients who died had significantly higher APACHE II score, thrombocytopenia, lower PaO2/FiO2 ratio, more comorbidities. Mean symptom to hospital admission and mean duration from symptom to initiation of oseltamivir was higher in non-survivors but was not statistically significant. Patients with rise in platelet count <15% from baseline had higher mortality of 54.28% (19/35) compared to patients who showed rise in platelet count >15% from baseline 13.88% (5/36). On multinomial cox regression analysis, APACHE II score >10, rise in platelet <15% from baseline and presence of comorbidities had higher risk of mortality (Table 2).

Table 2 Multinomial cox proportional hazard analysis for factors influencing mortality in patients with H1N1 pneumonia.

Sr.No	Variables	Hazard ratio (95%CI)	p value	Multinomial Hazard ratio (95%CI)	p value
1	APACHE II Score >10	4.83(2.01-11.57)	**0.000**	3.41(1.12-10.35)	**0.030**
2	Rise in Platelet count <15% from baseline	4.06(1.50-10.96)	**0.006**	4.47(1.39-14.36)	**0.012**
3	Comorbidities	2.27(0.973-5.33)	**0.05**	4.37(1.67-11.43)	**0.003**
4	Vasopressor Usage	4.33(1.89-9.40)	**0.000**	1.70(0.54-5.35)	0.358

Kaplan Meier analysis of APACHE II >10 and rise in platelet count <15% from baseline confirms significant association with mortality (Fig 2 and 3 respectively).
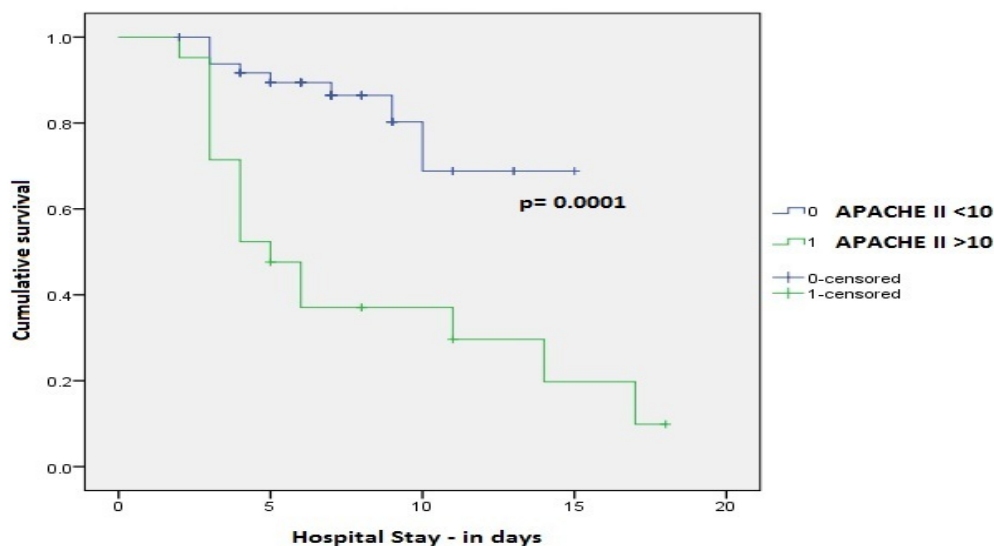

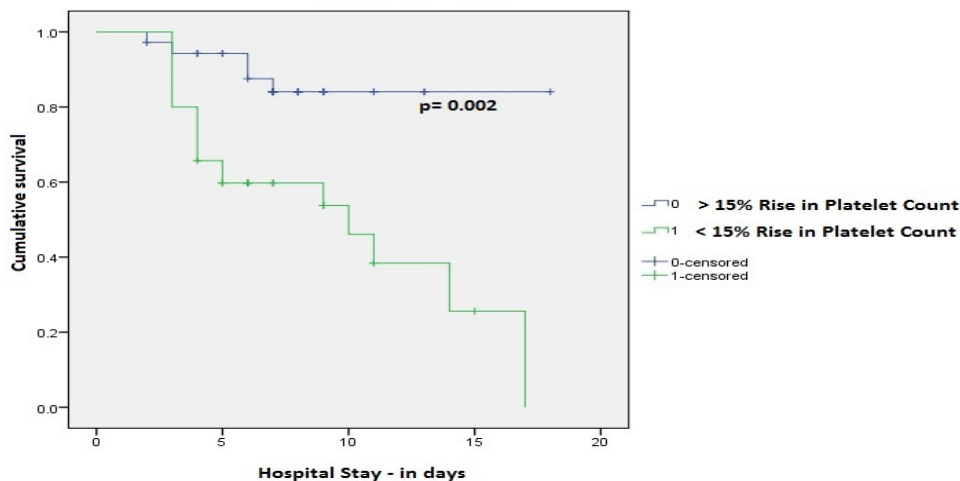**Fig. 2 Cumulative survival rates of patients with H1N1 pneumonia with APACHE II Score at admission**

Fig. 3 Cumulative survival rates of patients with H1N1 pneumonia with rise in platelet count <15% from baseline.

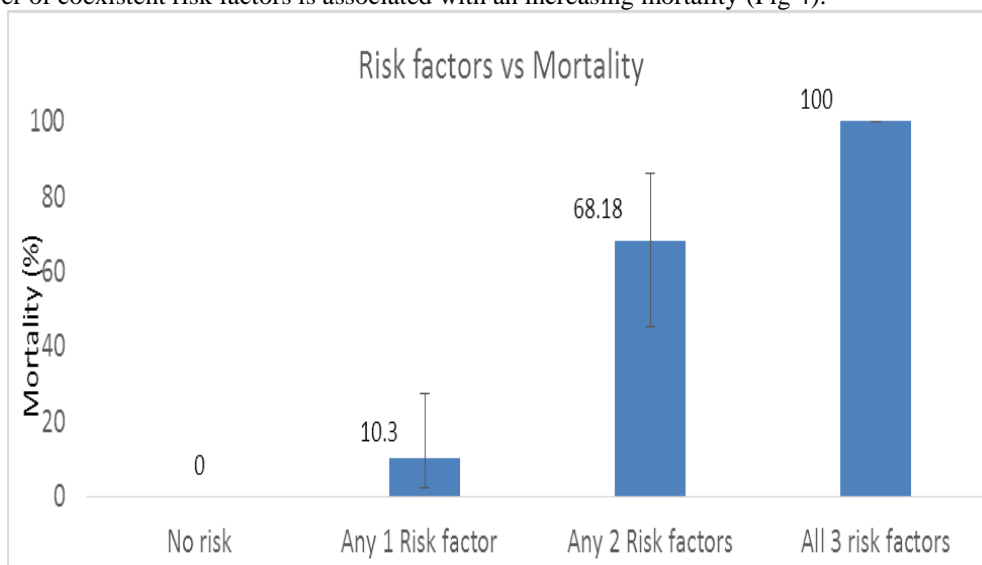Higher number of coexistent risk factors is associated with an increasing mortality (Fig 4).

Fig.4 The influence of higher number of risk factors on mortality (in proportions). Risk factors are APACHE II score >10 at admission, Rise in platelet count <15% from baseline, presence of Comorbidities (only significant risk factors identified in the multinomial cox proportional hazards model are included)

Follow-up at 3 months and 1 year: Forty-one patients showed no residual symptoms or activity limitation. Six patients were lost to follow up. Five patients could not perform spirometry according to American thoracic society (ATS) standards. Acceptable spirometry results were obtained for 36 subjects (Table 3). Status quo was maintained in patients with COPD and asthmatics with regard to their severity of disease, medication requirement and exacerbation frequency. There were no deaths after discharge.

Table 3 Spirometry results among Survivors at 3rd month and 1 year after discharge (N=36)

Sr.No		Spirometry at 3rd month		Spirometry at 1 year	
		Actual	Predicted	Actual	Predicted
1	FVC pre, mean(SD)	2.54(0.70)	91.94(12.3)	2.72(0.75)	98.53(14.14)
2	FVC post, mean(SD)	2.56(0.71)	92.22(13.04)	2.74(0.72)	99.94(14.66)
3	FEV1 pre, mean(SD)	2.03(0.57)	87.72(14.69)	2.23(0.64)	96.84(16.16)
4	FEV1 post, mean(SD)	2.12(0.60)	91.47(15.22)	2.31(0.63)	100.56(15.70)
5	FEV1/FVC % pred (post), mean(SD)	0.82(0.076)	99.25(8.73)	0.83(0.060)	100.91(6.57)

4. DISCUSSION

H1N1 continues to be important even after the 2009 pandemic due to a continuing epidemic in many countries including India (2014-16), affecting healthy young population with high mortality in those with severe disease. This study was conducted due to sparse data among Indians regarding factors predicting mortality after H1N1 pneumonia and no studies on long-term sequelae among H1N1 survivors. We observed that it affected a relatively young population (44.42±12.81 years) with high mortality among patients presenting to the Emergency room needing admission. Patients whose disease progressed to need ventilator support had a mortality of 87.5%. Important independent predictors of mortality in our study observed were improvement in platelets at 3rd day from the baseline <15%, APACHE II score >10 and presence of comorbidities. Follow up of survivors at 3rd month and 1 year revealed no residual symptoms, long-term radiological sequelae or loss of pulmonary function.

Platelets are important in host immune response, promoting pathogen recognition by different mechanisms. Viral infections activate platelets and causes increased platelet destruction by inducing antiplatelet antibody[24], FcγRII mediated interactions with immune complexes, by direct interaction with platelets, decrease platelet production by infecting megakaryocytes causing decreased maturation and apoptosis or by infecting hematopoietic stem cells causing growth deficient megakaryocyte colony forming units.[25] In H1N1 patients, virus adsorbs over platelets promoting IgG and complement deposition resulting in structural alterations and platelets destruction.[26,27] Animal studies confirm drastic reduction of platelets soon after infusion of influenza virus suspension after 1 hour and recovery usually starts by the end of first day and reaches normal levels by 2-3 days.[27] Our patients had low platelets at clinical presentation and had more than 5 days of clinical symptoms. Sustained thrombocytopenia may be a surrogate marker for prolonged viremia in these subset of sick patients. Many studies have confirmed thrombocytopenia at admission as an independent risk factor for mortality in critically ill patients and a risk for severe illness and organ dysfunction.[11,22] Several studies observed lower platelet counts among non-survivors versus survivors.[9–11] We observed lower mean platelet count in non-survivors compared to survivors (151±51x10^9/l vs 190±76.6x10^9/l p=0.05). A Spanish study showed lower survival rate (55% versus 92.5%) in acute respiratory failure patients with H1N1 and thrombocytopenia at admission.[16]

There are no studies on improvement in platelets on survival, which is a novel outcome measure in our study. A randomized double blinded study that compared viral shedding and titres in patients treated with placebo and oseltamivir (75mg and150mg) observed that by 3rd day viral titre almost reached zero in patients receiving either of the doses of oseltamivir.[28] Reduction in viral load leading to cessation of platelet destruction is the most likely reason for increase in platelet counts after anti-viral therapy. Oseltamivir could in addition, increase platelet counts by inhibiting sialidase as well as neuraminidase decreasing platelet surface sialic acid, both of which helps to reduce platelet clearance.[29,30] More than half of the subjects 54.28% (19/35) who had <15% improvement in platelet count from their baseline died compared to 13.88% (5/36) in patients with improvement in platelet count >15%. This could be an important marker for prognosis if confirmed in future studies in different populations.

APACHE II (Acute Physiology and Chronic Health Evaluation II) score is a severity of disease classification applied within first 24 hours of admission to ICU. Higher APACHE II scores indicates more severe disease and higher risk of death.[21] Most studies using APACHE II in H1N1 observed lower scores which should have lower mortality rates according to mortality prediction for APACHE II scores; 8% mortality for APACHE II score 5-9, 15% mortality for a score of 10-14 and 24% mortality for a score of 15-19. However, most studies have shown much higher mortality of nearly 40%, since most of these patients had ARDS. The Spanish study was an exception with a lower mortality of 20% which could be because of early presentation to hospital and treatment with mean duration of 1.2±0.42 days before initiation of oseltamivir, compared to 5.41±2.76 (present study), 5.8±2.7 days[10] and median of 6(4-8) days.[9] Though the APACHE II scores in the Spanish study(13.3±7) was much higher than in our patients (7.64±3.77), their mortality rates were nearly half of most other studies. This emphasizes the need for early treatment with oseltamivir to prevent adverse outcomes.

Comorbidities increases the risk for mortality in critically ill patients and form an important part of ICU scores like APACHE II and SOFA. We observed comorbidities to be an independent risk factor for mortality. Nelson confirmed that patients with comorbidities have higher initial viral load than without comorbidities (5.06±1.85 vs 3.62±2.13 log10 copies/ml).[31] Diabetes is among the most important comorbidities in patients with influenza and increases risk of hospitalization (three times), ICU admission (4-6 times)[32,33] and death.[34–37] Diabetics are found to have lower antiviral immunity due to impaired functions of NK cells, macrophages, neutrophils and lower levels of interferons.[38–40] Diabetics have systemic inflammation and oxidative stress that increases expression of Fcγ receptor type IIa (FcγRIIa) on platelets, the target sites for binding of H1N1 virus.[41] Diabetes increases risk of death in patients with H1N1.[42,43] Diabetes was the most common comorbidity in our study (24%) and an independent predictor of mortality in patients with H1N1.

There were not many studies on the continuing epidemic in India in 2015 evaluating factors predicting mortality in H1N1. We screened all patients presenting with

suspected H1N1 to the EMD thus limiting selection bias. We observed rise in platelet count <15% from baseline on the 3rd day to be an independent predictor of mortality which is a novel finding. There are no studies with both short-term (3 months) and long-term (1 year) follow-up of H1N1 patients except for few case reports. The results of this study are generalizable only to patients attending the emergency department in need of admission and not to subjects treated as outpatients. This study is done in a single tertiary care teaching hospital catering mainly to low and middle-income patients.

5. CONCLUSION
H1N1 continues in India with a high mortality rate. APACHE II scores >10, presence of comorbidities and a rise in platelet count <15% on the 3rd day of starting oseltamivir are associated with a higher risk of mortality. Early diagnosis and initiation of oseltamivir may improve treatment outcomes.

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