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To determine the difference in mean serum CPK level in H1N1 Positive and H1N1 Negative influenza like illness cases, SMS Hospital, Jaipur.

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Abstract
Background: *The great majority of individuals infected with the A (H1N1) virus experience uncomplicated influenza-like illness, with full recovery within 1 week, even without medical treatment, whereas a small subset of patients develops ever progressive disease. Number of Swine Flu Cases and Deaths in India in 2009 was 272 Cases and 981 death while in 2015 was 342 Cases & 2115 Death. The H1N1 virus outbreak had previously occurred India during the 2009 flu pandemic. The virus killed 981 people in 2009 and 1763 in 2010. The mortality decreased in 2011 to 75. It claimed 405 lives in 2012 and 699 lives in 2013. In 2014, a total of 218 people died from the H1N1 flu. [1,2]*
Material & Method: *This was a hospital based observational and comparative study on patients admitted to SMS Hospital with influenza like illness conducted in the Department of Medicine, S.M.S Medical College &Associated Group of SMS Hospitals, Jaipur (Rajasthan) from February 2015 to March 2016 after obtaining Ethical approval from institutional research ethics committee and written informed consent from all subjects. Each patient who was suspected clinically to be H1N1 positive was placed in one of three categories according to the guidelines provided by Ministry of Health and Family welfare in August, 2009.[3] All subject were treated by oral oseltamivir 75 mg bd, iv antibiotic, and symptomatically. Descriptive statistics will be used for clinical, demographic and lab data. Qualitative data will be analysed using chi square test and quantitative data using t test and Receiver Operator Characteristics Curve will be used for assess the optimal cut off value for serum creatine phosphokinase for diagnosis of H1N1 cases. Statistical significance will be set at $P < 0.05$.*
Result: *In present study, the mean serum CPK level has been determined to be significantly higher (757+649.7) in H1N1 positive cases as compared to H1N1 negative cases (69.3+13.8).*
Conclusion: *It can be concluded that our comparative study of Serum creatine phosphokinase level among H1N1 positive and H1N1 negative show that mean value of serum creatine phosphokinase level was significantly higher ($p<0.01$) in H1N1 positive cases .*

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I. Introduction

Humanity and epidemics share a long and eventful past. Just when we thought that we have seen the last of the Ebola outbreak, which was more of a scare in India, we are faced with another one i.e. the 'Swine Flu' scare; only that this time it is ominously real.

The influenza A (H1N1)virus contains a unique combination of gene segments that has not previously been identified in humans or animals[3,4]; thus, information on the clinical spectrum of illness, risk factors for severity among persons hospitalized for the treatment of A (H1N1) influenza and clinical management is helpful[4-1].

Primary viral pneumonia is the most common finding in severe cases[5], but secondary bacterial infections play a role in approximately 30% of fatal cases[6]. Hospitalized patients are often affected by other

medical conditions, such as diabetes and cardiovascular, neurological and pulmonary diseases.[4,5,6]

Number of Swine Flu Cases and Deaths in India in 2009 was 272 Cases and 981 death while in 2015 was 342 Cases & 2115 Death

Number of Swine Flu Cases and Deaths in Rajasthan in 2009 was 3032 Cases and 150 Deaths while in 2015 was 6597 Cases and 406 Death.

Number of Swine Flu Cases and Deaths at SMS Hospital Jaipur, Rajasthan in 2009 was 291 cases and 77 deaths while in 2015 was 636 cases and 132 Deaths.

In first 3 month of 2015 (up to 8th April) a total no of 6597 H1N1 Positive cases and 406 fatal cases reported in Rajasthan which was highest in India.[1]

Present study is "A comparative study of serum cpk level among H1N1 positive and H1N1negative influenza like illness patients hospitalized in the SMS hospital Jaipur Rajasthan this analysis our experience from a tertiary care institute admitting influenza like illness. This is observational and comparative study of reported cases admitted from February 15 to March 2016.

Creatine Phosphokinase (CPK OR CK)[7] catalyzes the reversible transfer of phosphate groups between creatine and phosphocreatine as well as between ATP and ADP and resides in skeletal muscle, heart muscle, and in the gastrointestinal tract. It enters the blood rapidly following damage to muscle cells and it's measurement of CPK levels still provides valuable differentiating diagnostic information**.**

ISOENZYME OF CREATINE KINASE

Isoenzyme	Electrophoretic mobility	Tissue of origin	Mean percentage in blood
MM (CK-3)	Least	Skeleton muscle + heart muscle	97-100
MB (CK-2)	Intermediate	Heart muscle	0-3
BB (CK-1)	Maximum	Brain	0-1

Diagnostic application:-

Total serum creatine kinase:-

Normal:-38-174IU/L in men

26-140IU/L in women

Ceratine kinase also increase in hyper-or-hypothermia, hypothyroidism, after normal vaginal delivery BB isoenzyme from myometrial contraction and Reye's syndrome.

II. Aims And Objectives

Primary Objective

- To determine the difference in mean serum CPK level in H1N1 Positive and H1N1 Negative influenza like illness cases, SMS Hospital, Jaipur.
- To estimate the proportion of raised serum creatinine phosphokinase in hospitalized H1N1 positive and H1N1 negative cases.

III. Material And Methods

Present study was conducted in the Department of Medicine, S.M.S Medical College &Associated Group of SMS Hospitals, Jaipur (Rajasthan) from February 2015 to March 2016. This was a hospital based observational and comparative study on patients admitted to SMS Hospital with influenza like illness. Ethical approval was obtained from institutional research ethics committee and written informed consent was taken from all subject.

Sample Size:

Sample size was calculated to be 33 subjects in each of the 2 groups assuming the difference in mean serum CPK level among H1N1 positive and H1N1 negative influenza like illness patient to be 270 IU/L and standard deviation to be 380 (as per seed article). Hence for study purpose 35 subjects will be taken in each of the two group.

Method of collection of data:

This study was a observational and comparative study done at Sawai Man Singh Medical College, Jaipur in the Department of Medicine between 1st February 2015 to March 2016. Nasal/Throat swabs were taken in Viral Transport Media (VTM), after collecting relevant clinical information and epidemiological information such as age, gender, underlying disease, travel history, close contact with confirmed case etc. from the patient.

RNA was extracted using QIA amp viral RNA Mini Kit (Qiagen GmbH, Germany) and Real time RT-PCR was used for clinical confirmation of the presence of H1N1, at our advanced Microbiology Research Lab .

Inclusion criteria

- Case All patients positive nasopharyngeal swab for H1N1 influenza by RT- PCR method who were admitted in SMS hospital Jaipur from February 2015 to March 2016 included in the Control Next consecutive patient who will be negative for RT-PCR

Exclusion Criteria

- Patient having other conditions that can cause increased Creatine Kinase level like acute myocardial infarction (heart damage) or skeletal muscle damage , myopathy.
- Who do not provide consent

CLINICAL PROTOCOL

Each patient visiting either the swine flu OPD or the swine flu ward, who was suspected clinically to be H1N1 positive was placed in one of three categories according to the guidelines provided by Ministry of Health and Family welfare in August, 2009.

Category A

- Mild fever plus cough / sore throat with or without body ache, headache, diarrhea and vomiting.
- No testing for H1N1 is required in such patients

Category B

i. Above signs and symptoms plus high grade fever and severe sore throat.
ii. Addition of above symptoms and signs plus one or more of the following conditions: Children less than 5 years Pregnant women Age above 65 years Having lung, heart, liver or kidney diseases, blood disorders, diabetes, neurological disorders, cancer and HIV, Long term cortisone.

Category C

- In addition to symptoms and signs of A and B if patients have one or more of the following: Breathlessness, chest pain, drowsiness, low BP, sputum mixed with blood, bluish discolouration, irritability among small children, refusal to accept feeds & worsening of underlying chronic conditions.
- All the patients fulfilling the selection criterion were selected for the study and consent obtained.
- All subject were treated by oral oseltamivir 75 mg bd, iv antibiotic, and symptomatically. Critically ill patients not maintaining oxygen saturation were put on invasive or non invasive mechanical ventilation. The patients were followed from the day of admission till their discharge from the hospital.

Statistical Methods

Descriptive statistics will be used for clinical, demographic and lab data. Qualitative data will be analysed using chi square test and quantitative data using t test and Receiver Operator Characteristics Curve will be used for assess the optimal cut off value for serum creatine phosphokinase for diagnosis of H1N1 cases. Statistical significance will be set at $P < 0.05$.

IV. Observations

Table 1: Comparison of CPK level in H1N1 positive and Negative groups

Group	N	Mean CPK (U/L)	Std. Deviation (U/L)
H1N1 negative	35	69.3	13.8
H1N1 positive	35	757.5	649.7

t = -6.241 with 68 degrees of freedom; P <0.001

Above table shows that mean CPK level was higher in H1N1 positive patients (757.5 U/L) as compared to H1N1 negative patients (69.3 U/L). Application of t test revealed that this difference was statistically significant (P<0.001).

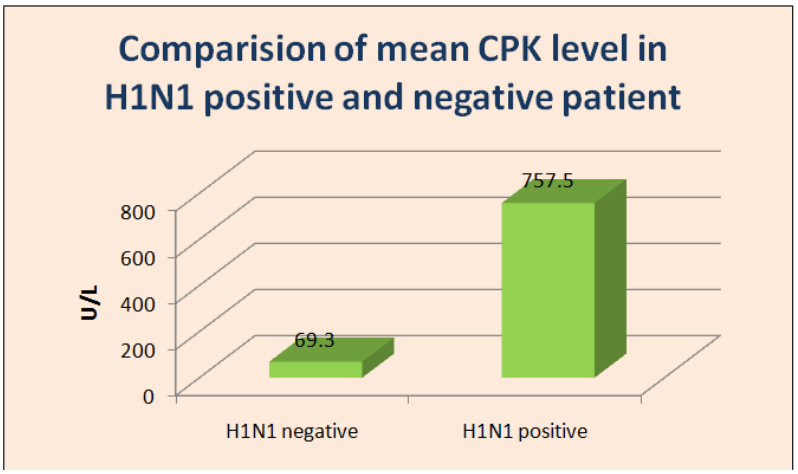
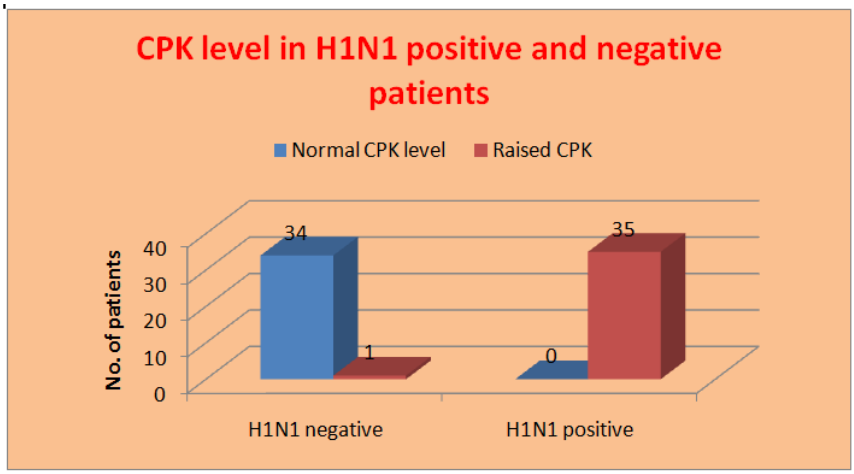


Table 2: Distribution of study subjects according to their CPK level

CPK level	H1N1 negative		H1N1 positive		Grand Total	
	N	%	N	%	N	%
Normal	34	97.1	0	0	34	48.6
Raised	1	2.9	35	100	36	51.4
Grand Total	**35**	100.0	**35**	100.0	**70**	100.0

Chi-square = 62.279 with 1 degree of freedom; P < 0.001

Above table reveals that all of the H1N1 positive patients had raised CPK level while only one patient in H1N1 negative patient had raised CPK level and this difference was found to be statistically significant (P<0.001).



V. Discussion & Results

Influenza A H1N1 is a highly contagious pathogen which made headlines in 2009, as the so called swine flu. Rajasthan was one of the foremost affected states bearing the frontal attack in which majority of deaths occurred early and in the young. Total number of 636 Influenza A H1N1 cases and 132 deaths (case fatality ratio-20.75%) were reported in SMS hospital Jaipur in recent outbreak. During pandemic 2009-2010 case fatality ratio at SMS Hospital J, Rajasthan was reported as 26.46%. The low case fatality ratio in the recent outbreak indicates that general population benefit from cross protection of earlier flu pandemic.

In present study, the mean serum CPK level has been determined to be significantly higher (757+649.7) in H1N1 positive cases as compared to H1N1 negative cases (69.3+13.8).

This results are in concordance with the results of Hashemian, et al. who observed the mean serum CPK level in 46 patients hospitalized with confirmed swine flu(H1N1) pneumonia was high (638.6±696.1).

All these findings confirm that the CPK level is significantly higher in H1N1 positive cases and also various other biochemical abnormalities.

VI. Conclusion

The A (H1N1) virus is still active two year after the 2009 pandemic in the Western Rajasthan region of India. The incidence and mortality in recent outbreak of H1N1 influenza was higher in young individual especially during the winter months.

It can be concluded that our comparative study of Serum creatine phosphokinase level among H1N1 positive and H1N1 negative show that mean value of serum creatine phosphokinase level was significantly higher ($p<0.01$) in H1N1 positive cases .

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