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Assessment of morbidity and mortality by influenza A (H1N1) among pregnant women in Western Rajasthan, India

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Abstract

Background: Influenza has a major impact on public heath, First outbreak of influenza as a pandemic declared by WHO in June 2009. The present study determines the morbidity and mortality of influenza A (H1N1) by real time reverse transcriptase polymerase chain reaction (RT-PCR) method in pregnant women.

Objective: To study the morbidity and mortality of influenza A (H1N1) by RT-PCR Method in pregnant women.

Materials and Methods: 702 respiratory samples (nasopharyngeal swabs) of reproductive age group (17 to 45 yrs) women were collected from Dr S. N. Medical College & associated group of Hospitals from January 2015 to March 2015. The samples were collected and processed as per World Health Organization (WHO) guidelines. Viral RNA was extracted and one-step RT-PCR was performed to detect influenza A (H1 and N1) virus.

Results: Out of 702 samples 230 (32.7%) were pregnant and 472 (67.3%) were non pregnant women. Among pregnant 63 (27.4%) and among non pregnant 120 (25.4%) were positive for influenza A (H1N1) and 18 (28.5%) pregnant women died due to influenza A (H1N1) infections.

Conclusion: It was observed that influenza A (H1N1) was prevalent in pregnant women in western Rajasthan (Jodhpur). We emphasize on early detection and monitoring of influenza in pregnant women which will be helpful in patient management who are at risk for influenza complications.

Key words: influenza A (H1N1), pregnant women, RT-PCR, Rajasthan

Introduction

The Influenza A H1N1 pandemic (A H1N1) occurred between June 2009 and August 2010 [1]. The outbreak became widespread throughout India. On 12th February, 2015, Rajasthan was declared an epidemic for swine flu.[2]

Pregnant women are especially at high risk for development of complications of H1N1 influenza A [3] leading to higher morbidity and mortality rates as compared to non-pregnant women. [4]

Relevant immunologic alterations also occur during pregnancy, with a shift away from cell-mediated immunity towards humoral immunity. This shift can render pregnant women more susceptible to, or more severely affected by, certain viral pathogens, including influenza.[5]

Though Influenza A (H1N1) infection in pregnancy is mild in nature, but considering the high rates of morbidity & mortality associated with it, emphasis should be laid on early diagnosis and treatment.[6]

The CDC recommends immediate antiviral treatment of pregnant women with suspected or confirmed H1N1 influenza, preferably within 48 h after the onset of symptoms.[7] Influenza viruses are known to cause frequent epidemics and periodic pandemics, and are unique with regard to their antigenic variability, seasonality and impact on general population. Influenza like illness (ILI) screening and surveillance programme are critical in tracking the activity, especially of influenza viruses, across seasons. Since the critical differential diagnosis is difficult, such programmes become imperative in the control and management of respiratory viral disease outbreaks.[8]

Influenza virus is an enveloped RNA virus of the Orthomyxoviridae family. It is endowed with an inherent capacity for genetic variation and is based on two important features; (i) the presence of a segmented genome, with eight RNA segments that are genetically independent of each other and (ii) a high rate of mutation, especially in the surface heamgglutinin (H) and neuraminidase (N) proteins.[9]

Aims & Objectives

To study the morbidity and mortality of influenza A (H1N1) by real time reverse transcriptase polymerase chain reaction (RT-PCR) method in pregnant women.

Material and methods

The study was conducted in Microbiology department of Dr. S. N. Medical College Jodhpur Rajasthan from January 2015 to march 2015 (3 month period).

A total of 702 clinical samples of reproductive age group (17 to 45 yrs) women were collected from OPD and IPD patients of Dr. S.N. Medical College & attached group of hospitals, Jodhpur Rajasthan showing Influenza like sign and symptoms. Clinical samples were collected taking aseptic precautions in a viral transport medium (VTM), and immediately transported to microbiology laboratory in a Styrofoam box containing ice packs or vaccine carrier box.

Processing of samples and identification:

As per the laboratory criteria for diagnosis of influenza specimens suggested by WHO, the RT-PCR protocol was adopted [10]. The throat swabs in VTM samples were processed in a Biosafety level II Cabinet and divided into three aliquots and stored at -80°C in deep freezer. Processing of samples was done according to the CDC standard protocol [11]. Use of appropriate biosafety measures and personal protection equipment (PPE) were as per CDC's and WHO laboratory biosafety guidelines[12, 13]. RNA extraction kit (Qiagen, USA) and primers (ABI, USA) were also according to the CDC protocol [11].The viral RNA was extracted from clinical samples using the spin column based QAIamp® Viral RNA mini kit (Qiagen GmbH, Hilden, Germany) as per the manufacturer's instructions. Primers and probes were custom synthesised from Applied Biosystems (AB), USA, for influenza type A,(H1) Swine A,and Swine H1. Primers and probes were CDC approved. Real-time reverse transcriptase polymerase chain reaction (rRT-PCR) was performed by using a Step One Real Time PCR instrument (AB) with an rRT-PCR kit containing 2 × PCR mix and SuperScript™ III RT/Platinum Taq Mix (Enzyme mix) from Invitrogen (CA, USA), and influenza A, Swine A and swine H1, primers and probes. A master mix of 20 µL was prepared in a 48 well PCR plate and 5 µL of RNA

template was added and the plates were placed in the AB Step One Real-time PCR instrument using cycling conditions of 50°C for 30 min of reverse transcription followed by Taq inhibitor inactivation at 95°C for 10 min and PCR amplification (45 cycles) at 95°C for 15 sec and at 55°C for 30 sec.

Results

A total of 702 clinical samples of reproductive age group (17 to 45 yrs) women with ILI symptoms were collected, out of which 230 (32.7%) were pregnant and 472 (67.3%) were non pregnant women. Among pregnant 63 (27.4%) and among non pregnant 120 (25.4%) were positive for influenza A (H1N1) and 18 (28.5%) pregnant women died due to influenza A (H1N1) infections. Influenza-like illness (ILI) is defined by the Center for Disease Control (CDC) as fever (temperature ≥ 100°F or 37.8°C) and either the presence of cough or sore throat in the absence of any known cause [14].

Symptomatically acute respiratory illness like nasal discharge, cough, fever, breathlessness and sore throat were prominent symptoms in pregnant women of ILI. Other symptoms like headache, fatigue, vomiting and diarrhea were also present.

After 2009 pandemic of influenza A (H1N1) Virus we compared total pregnant women samples and positive H1N1 pregnant women samples processed in Microbiology laboratory of Dr. S. N. Medical College Jodhpur Rajasthan by RT-PCR Method in last 6 years (2010 to 2015).

Table: 1 Total pregnant women and H1N1 positive pregnant women cases in different years.

Year	Total pregnant women	H1N1 positive Pregnant women
2010	160	38 (23.7%)
2011	38	10 (26.3%)
2012	14	3 (21.4%)
2013	174	45 (25.8%)
2014	8	0 (0%)
2015	230	63 (27.4%)

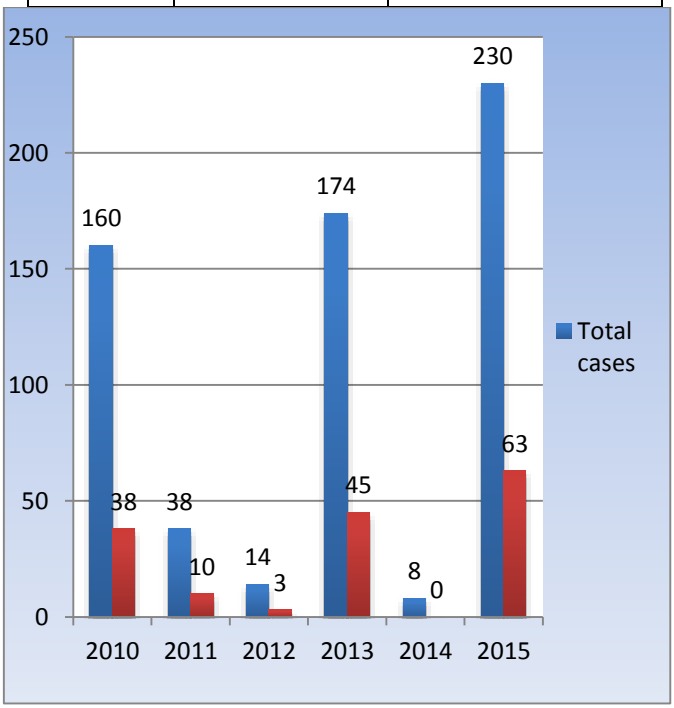

Figure : 1 showing total pregnant women and H1N1 positive pregnant women cases in different years.

Discussion

The clinical importance of the early identification of influenza was underscored by our recent surveillance data. Out of the total 230 suspected influenza samples in pregnant women, 63 (27.4%)) were positive for influenza A (H1N1) and 18 (28.5%) patients died due to influenza A (H1N1) infection.

There is considerable evidence that pregnant women are at increased risk for severe illness from influenza infection. Cardiopulmonary adaptive changes occurring in pregnancy, such as increased cardiac rate and stroke volume and reduced pulmonary residual capacity, may increase the risk of hypoxemia and contribute to the observed increased severity of influenza.[15]

Various study have proven that inactivated influenza vaccine given to pregnant mother that reduce the influenza illness in mother and infants both. K. Zaman et al study showed that inactivated influenza vaccine to pregnant mother reduced influenza illness by 63% in infants up to 6 months of age and averted approximately a third of all febrile respiratory illnesses in mothers and young infants. Maternal influenza immunization is a strategy with substantial benefits for both mother and infant.[16]

Our study constituted influenza A (H1N1) positivity among pregnant women to be 27.4% which was almost similar to study of Pramanick et al [17] (25.3% of pregnant/puerperal women) and Mathur et al [18] (23.4% of pregnant women).

In our study, mortality rate among H1N1 positive pregnant women was 28.5% in accordance with study of Jamieson DJ et al [19] who showed mortality rate of 28% and Harris JW.et al [20] who showed 27% deaths among pregnant women with H1N1 influenza infection.

We compared last 6 years data (from 2010 to 2015) and analysed that maximum number of pregnant women samples (230) came in year 2015 and maximum positive cases 63 (27.4%) for influenza A (H1N1) came in year 2015 while minimum samples 8 and minimum positive 0 (0%) for swine H1N1 was came in year2014.

The increased rates of detection of influenza cases has occurred over last 6 years due to increased awareness of patient to influenza-like illness, promptness in attending swine flu OPDs & rapid diagnosis of influenza A (H1N1) virus.

Conclusion

Influenza in pregnancy is a significant and under-appreciated public health problem. Its substantial morbidity and mortality impact can be mitigated by education of women and their physicians as well as vaccination and use of available preventive and therapeutic modalities. We emphasize on early detection and monitoring of influenza in pregnant women which will be helpful in patient management who are at risk for influenza complications.

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