SEROPREVALENCE OF PANDEMIC H1N1 INFLUENZA AMONG PREGNANT WOMEN IN KERALA, INDIA

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ABSTRACT

BACKGROUND

Pandemic influenza affected all sections of the society with maximum involvement among younger population. Pregnant females were at high risk for severe disease and complications.¹ This study was conducted to estimate the seroprevalence of pandemic H1N1 influenza among pregnant women in Kerala.

MATERIALS AND METHODS

A cross sectional study was conducted in three badly affected districts of Kerala during the post-pandemic period. Sample size was calculated with prevalence of 32%,² α error 5%, relative precision 20% and design effect of 1.5. Univariate analysis was done and proportions were calculated. Blood samples were collected from 355 pregnant women who participated in the study. ELISA, using a monoclonal antibody (mAb) against influenza A Haemagglutinin protein (HP) was done to detect antibodies against influenza.

RESULTS

Two third of the study population were housewives, high school educated and socioeconomically in the lower class. 98.3% of them were ELISA positive indicating that almost all of them were exposed to pandemic H1N1 influenza during the pandemic period.

CONCLUSION

Most of the pregnant women developed protective antibodies against pandemic H1N1 influenza, hence got public health relevance.

KEYWORDS

Seroprevalence, Pandemic H1N1 Influenza, ELISA.

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BACKGROUND

Pandemic H1N1 influenza affected millions of people in the world and caused considerable morbidity and mortality among general public. This pandemic was caused by a novel virus of swine origin resulting in the occurrence of the first pandemic in 40 years.³ In Kerala, there were 3322 confirmed cases and 131 deaths in the pandemic period, and this is expected to continue for few seasons after the pandemic was called off in November 2010 till most of the people acquire immunity against it.⁴ In Kerala, most of the cases were reported from southern districts and it occurred among all sections of the society with uniform distribution among all age groups.⁵

Pregnant women represent the active young population of the community and they were exposed to H1N1 during the pandemic period during their pre-pregnancy period. Pregnant status increases the risk for severe disease 4-5 times and this risk is highest in the third trimester.¹ This is because mother's body is rapidly changing, especially the cardiovascular, respiratory and immune systems.

Influenza in pregnancy is also associated with an increased risk of adverse pregnancy outcomes^{6,7}, such as spontaneous abortion, preterm birth, and foetal distress. To know the extent of spread of pandemic H1N1 influenza in the community, this study was carried out during the post-pandemic period with the objective to estimate the seroprevalence of pandemic H1N1 influenza among pregnant women in Kerala.

MATERIALS AND METHODS**Study Design**

Hospital based cross sectional study was conducted to find out the seroprevalence of pandemic H1N1 influenza among pregnant women in Kerala.

Study Setting

Kerala was one of the badly affected states during the pandemic H1N1 influenza in India.⁸ This study was carried out in three out of 14 districts of Kerala namely Thiruvananthapuram, Kollam and Pathanamthitta. Study participants recruited from all general, district and taluk hospitals in these districts formed the sampling unit. A total of 38 institutions were included in the study.

Study Subjects and Study Period

Pregnant women attending antenatal clinics were recruited for the study. In Kerala, the spread of pandemic H1N1 influenza occurred during the years 2009 and 2010. This study was conducted during the post-pandemic period from May to September 2011.

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Sample Size and Sampling Technique

Seroprevalence among general population varies from 20 % - 40% during pandemics.⁹ Expecting the same prevalence among pregnant women, sample size was calculated with prevalence of 30%, α error 5%, β error 20% and design effect of 1.5, sample size required for the study was estimated to be 350. From each sampling unit, 10 eligible people were recruited resulting in a final sample size of 355.

Ethical Consideration

Study was carried out after obtaining clearance from institution research committee and human ethics committee. Written informed consent was also obtained from each of the participant after explaining the purpose of the study. Study was conducted in accordance with the standards of ethics committee.

Data Collection

Study was conducted after obtaining permission from the heads of the institution. From each hospital either a staff nurse or a laboratory technician was identified and one day training was given to them for data collection. Pregnant women were recruited from a total of 38 secondary level care government hospitals in three selected districts of Kerala. All pregnant women seeking antenatal care from these hospitals constituted the study population and from each hospital ten participants were recruited for the study by consecutive sampling technique.

After obtaining written informed consent from each study participant, information regarding sociodemographic factors, pregnancy related factors namely gestational age and parity were collected using pre-tested structured questionnaire. From each participant 3-5 mL of blood was collected and safely transported at 4-8°C to testing laboratory for serological analysis.

H1N1 virus possesses haemagglutinin, the antigenic surface protein which mediates the entry of virus in to host cell

and this is the most important antigen against which neutralising antibodies are directed.¹⁰ In this study, ELISA using a monoclonal antibody (mAb) against influenza A Haemagglutinin protein (HP) - A/California/07/2009 was used to detect antibodies against influenza. Where H1N1 antigen was used as positive control, H1N1 negative human serum as negative control and patient serum were diluted from 1:10 to 1:2560. Wells with serum and positive control turn blue as Anti-H1N1 primary antibody recognises and binds specifically to H1N1 antigen and Horseradish peroxidase (HRP) - labelled secondary antibody recognises and binds specifically to anti-H1N1 primary antibody and will not wash away, HRP will oxidise the enzyme substrate, which will result in a blue colour. Wells with negative control will remain colourless. If the well with the samples at a dilution of 1:40 or more turns blue, it indicates the presence of H1N1 antibody. If the well remains colourless, then the H1N1 antigen is not present and the ELISA result is read as negative.

RESULTS

A hospital based cross sectional study was carried out to find out seroprevalence of pandemic H1N1 influenza among pregnant women in three districts of Kerala during post-pandemic period. Study participants were recruited from all secondary level health care institutions in public sector. Of the 355 study participants, 51% (n=181) were from Ernakulam, 30% (n=106) from Thiruvananthapuram and 19% (n=68) from Kollam. Almost half the study population was in 21-25 year age group, Seventy two percent of the study participants completed high school education and 77% were unemployed. Socioeconomic status was assessed using Kuppaswamy's socioeconomic status scale¹¹ based on education, occupation and income. Three fourth of the population (72.9%) belonged to poor socioeconomic status. District wise distribution of sociodemographic factors of 355 participants are given in Table 1.

Study Variables	Thiruvananthapuram (n=106)		Kollam (n=68)		Ernakulam (n=181)		Total (n=355)	
Age in Years	Number	%	Number	%	Number	%	Number	%
18-20	18	17.0	9	13.2	18	9.9	45	12.7
21-25	57	53.8	29	46.6	76	42.0	162	45.6
26-30	22	20.8	23	33.8	62	34.3	107	30.1
31 and above	9	8.5	7	10.3	25	13.8	41	11.6
Education								
Below SSLC	37	35	39	57	22	12	98	28
Above SSLC	69	65	29	43	159	88	257	72
Occupation								
Unemployed	76	71.7	60	88.2	124	68.5	260	77.3
Employed	30	28.3	8	11.8	57	31.5	95	22.7
Socioeconomic Status (SES)								
Low SES	85	80.2	54	79.4	120	66.3	259	72.9
Middle & Upper SES	21	19.8	14	20.6	61	33.7	96	27.1

Table 1. District Wise Sociodemographic Characteristics about Study Participants

Of the 355 participants, 89 % (n=314) were either primigravida (49%) or second gravida (39.5%). For 48.5% (n=172) of them, this was their first pregnancy. Forty four percent of pregnant women were in their second trimester of pregnancy (Table 2).

Study Variables	Thiruvananthapuram (n=106)		Kollam (n=68)		Ernakulam (n=181)		Total (n=355)	
Gravida	Number	%	Number	%	Number	%	Number	%
One	53	50.0	35	51.5	86	47.5	174	49.0
Two	41	38.7	27	39.7	72	39.8	140	39.5
Three and above	12	11.3	6	8.8	23	12.7	41	11.5
Parity								
Zero	54	50.9	35	51.5	83	45.8	172	48.5
One	47	44.4	28	41.1	83	45.8	158	44.5
Two	5	4.7	5	7.4	15	8.4	25	7.0
Gestational Age								
<6 weeks	3	2.8	6	8.8	7	3.9	16	4.5
7-12 weeks	27	25.5	21	30.9	40	22.1	88	24.8
13-28 weeks	37	34.9	35	51.5	84	46.4	156	44.0
29-37 weeks	39	36.8	6	8.8	50	27.6	95	26.7

Table 2. Information Regarding Pregnancy

H1N1 infection rate was high among pregnant ladies compared to general population as revealed by CDC studies done during the initial phase of the outbreak.¹² Present study showed that 98.3% (n=349) of pregnant women sero-converted (table-3) and only 6 participants remained seronegative to pandemic H1N1 influenza indicating that almost all of the population were infected during the outbreak of H1N1 pandemic influenza in Kerala.

History regarding exposure to influenza like illness (ILI) during current pregnancy and exposure to H1N1 during pandemic period was collected during the study. 15.2% of the participants were exposed to ILI during the pandemic period.

Out of 355 pregnant ladies who participated in the study only 0.8% (n=5) had history of contact with confirmed pandemic H1N1 influenza and 1.9% (n=7) of them already received vaccination against Pandemic influenza.

ELISA Result	Thiruvananthapuram	Kollam	Ernakulam	Total
ELISA Positive	106	62	181	349
ELISA Negative	0	6	0	6
Seroprevalence	100%	91%	100%	98.3%
Total	106	68	181	355

Table 3. Seroconversion Status of Study Participants

DISCUSSION

This study was done among pregnant ladies from three districts of Kerala and serology showed that 98% of them had antibodies against pandemic influenza. This supported by a systematic review and meta-analysis of 55 studies conducted by Luis Furuya-Kanamori et al reported that seroprevalence varied from 13-91%.¹³

In this study, 15.7% of pregnant women had history of exposure to influenza like illness during last pregnancy, less than one percent had exposure to proven H1N1 case and 2% received vaccination against H1N1 pandemic influenza. This indicates that most of the pregnant ladies had subclinical infection during pandemic influenza period without any clinical manifestation. Pregnant ladies represented young population generally exposed to H1N1 pandemic influenza at the time of outbreak so they might have got exposed to H1N1 at that time. These people actually represent a cross section of the general population, high seropositivity among these groups indicates that most of the population in the study area might have had clinical or subclinical infection during the outbreak season. Epidemiological studies revealed that

protection against infection lasts for many years¹⁴ indicating that most of the population in the study area are immune to further outbreaks of pandemic H1N1 influenza.

CONCLUSION

Pregnant women who participated in the study were in the age group 18 to 40 years, most of participants were of 21-30 years, most of them were unemployed and belonged to low socioeconomic status, half of them were primigravida with parity zero or one. 44% of the participants were in their second trimester of pregnancy. In contrast to 10 to 20% prevalence in other studies conducted in India and other countries,^{15,16} very high proportion (98.6%) of pregnant women were sero-converted during the pandemic in Kerala.

Pregnant ladies who represent young population might have got exposed to H1N1 at that time. These people actually represent a cross section of the general population, high seropositivity among these groups indicates that most of the population residing in Thiruvananthapuram, Kollam and Ernakulam districts might have had clinical or subclinical infection during the outbreak season.

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