Occurrence and Protective Level of Influenza Infections Using Serology in Patients with COPD in Vaccination Study

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Objective : *To investigate the prevalence, occurrence and protective level of influenza infections using serology in patients with chronic obstructive pulmonary disease (COPD) during a one-year influenza vaccination study.*

Material and Method : *A total of 123 patients with COPD were enrolled during the period of 1997 to 1998. There were 61 patients in the vaccine group and 62 patients in the placebo group with a mean age $\pm$ SD of 67.6 $\pm$ 8.0 and 69.1 $\pm$ 7.5, respectively. The vaccine was composed of influenza A/Texas/36/91 (H1N1), A/Nanchang/933/95 (H3N2) and B/Harbin/07/94 strains. Antibodies to influenza viruses were detected by hemagglutination inhibition (HI) test using antigens of vaccine strains.*

Results : *The incidence of influenza proven by serological examination was 22/123 (17.9%) cases. Among 17/62 (27.4%) influenza cases in the placebo group representing natural infections, 3 (17.6%) were diagnosed as A (H1N1), 8 (47.1%) as A (H3N2), 3 (17.6%) as type A, 1 (5.9%) as type B and 2 (11.8%) as untypeable viruses. The 8.2% of influenza cases found in the vaccine group was significantly lower than 27.4% of that in the placebo group (Chi-square test, p = 0.01). The protection rate of influenza vaccination was 71%. Among 23 acute blood samples from 22 influenza cases, the titers ranged from < 10 to 20 corresponding to its type/subtype.*

In the vaccine group, 5 influenza cases occurred at 7, 7, 10, 11 and 11 months after vaccination. The HI antibodies to influenza A (H1N1), A (H3N2) and B viruses at titers of $\geq$ 10 vs $\geq$ 40 were 50.4% vs 21.9%, 54.5% vs 28.5% and 17.9% vs 4.1%, respectively.

Conclusion : *The findings indicated that from 1997 to 1998, the occurrence of influenza as natural infection was 27.4%. Influenza A (H3N2) was more frequently prevalent than A (H1N1) and B viruses. The influenza vaccination in COPD patients was effective. The protective HI antibody titers were $\geq$ 40. The patients without protective HI antibody to A (H1N1), A (H3N2) and B viruses were 78.1%, 71.5% and 95.9%, respectively. Such patients were considered to be at high-risk for influenza and recommended to have vaccination.*

Keywords : *Influenza, Prevalence, Occurrence, Incidence, Protective antibody, Diagnosis, Vaccine*

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Infections caused by influenza viruses lead to morbidity and mortality, particularly among elderly persons and patients with chronic pulmonary conditions[1,2]. Two options are available for the control of influenza virus infections: vaccination or therapy with influenza specific antiviral agents. Annual influenza vaccination is recommended for these high-risk persons[3].

There are two major types of influenza viruses, types A and B, which are responsible for disease in humans. Diseases resulting from influenza virus infection range from mild respiratory illness to fatal pneumonia. Influenza viruses contain a genome of single-stranded RNA segments, two of which encode

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two envelope proteins, hemagglutinin (H) and neuraminidase (N)[4]. The direct method of laboratory diagnosis of influenza is provided by virus isolation or antigen detection. The serology for detecting antibodies to H molecules[5] is an acceptable method of diagnosis.

Recovery from influenza virus infection involves both humoral response to the two surface glycoproteins H and N, and cell-mediated response to the internal proteins. Antibodies to H neutralize viral infectivity and are primarily responsible for resistance to infection, while antibodies to N represent a partial protective ability[4].

The objective of this study was to investigate the prevalence, occurrence and protective HI antibody level of influenza infections in patients with chronic obstructive pulmonary disease (COPD) during a one-year influenza vaccination study.

Material and Method

Study population

One hundred and twenty-three patients with chronic obstructive pulmonary disease (COPD) who attended the COPD clinic at Siriraj Hospital, Bangkok, Thailand, were enrolled in the one-year study during the period of June 1997 to November 1998. The mean age $\pm$ SD values of 61 patients in the vaccine group and 62 patients in the placebo group, were 67.6 $\pm$ 8.0 and 69.1 $\pm$ 7.5, respectively.

Vaccines

The vaccine used was the purified trivalent split-virus vaccine manufactured by Pasteur Merieux from France. Each dose of vaccine contained influenza A/Texas/36/91 (H1N1), A/Nanchang/933/95 (H3N2) and B/Harbin/07/94. Vitamin B1 was used as placebo.

Specimen collection

Ten ml of venous blood were collected from each patient at the first visit at which time the first dose of vaccine or placebo injection for baseline antibody (B1) detection was given. The samples were then collected at the following times: 1 month after the first dose (the second dose of injection) (B2), 2 months (B3), 6 months (B4) and 1 year (B5) after the first injection.

Laboratory diagnosis of influenza in patients with acute respiratory illness

When a patient developed acute respiratory illness (ARI), paired blood samples were collected at the initial visit (acute serum) and at 4-6 weeks afterward (convalescent serum). The samples were tested using a hemagglutination inhibition (HI) test for detecting influenza antibodies.

Hemagglutination inhibition (HI) test

Details of the procedure for the HI test have been described elsewhere[6], according to the protocol for HI testing set by the World Health Organization. Briefly, serum non-specific inhibitor was treated by receptor destroying enzyme from *Vibrio cholerae* (Denka Seiken, Japan) overnight at 4°C followed by inactivation at 56°C for 30 minutes. Non-specific agglutinator was removed by absorption with 50% chicken red blood cells. The antigens used as vaccine strains in the test were provided by Pasteur Merieux, Lyon-France. A serum which gave a positive result at a dilution of $\geq$ 1:10 was considered to have HI antibody. Serodiagnosis of influenza using HI was based upon demonstration of a 4-fold or greater increase in antibody titer or seroconversion.

Statistical analysis

The protection rate of vaccine was measured by calculating the influenza cases using the following formula:

$$\frac{\text{influenza cases amid non-vaccinated} - \text{influenza cases amid vaccinated}}{\text{influenza cases amid non-vaccinated}} \times 100$$

Data were analyzed by EPI INFO version 6.0. Comparison of influenza cases between the vaccine and placebo groups was performed by the Chi-square test. The level of critical significance was assigned at $p < 0.05$.

Results

Occurrence of influenza among COPD patients during a one-year influenza vaccination trail

The incidence of influenza using serodiagnosis is shown in Table 1. A total of 123 patients with COPD were enrolled in the present study. During a one-year influenza vaccination trail, the episodes of clinical presentations of ARI in the vaccine and placebo groups, were 111 (43%) and 145 (57%), respectively. The incidence of influenza virus infections proven by serology was 22/123 (17.9%). In vaccinated patients, the infections of influenza A(H1N1) and A(H3N2) viruses were found in 2/5 (40%) and 3/5 (60%) cases, respectively. Of 17/62 (27.4%)

influenza cases in the placebo group, 3 (17.6%) of influenza A (H1N1), 8 (47.1%) of A(H3N2), 3 (17.6%) of type A, 1 (5.9%) of type B and 2 (11.8%) of untypeable viruses.

Seroprotective level for influenza virus infections

The HI antibodies to influenza viruses in 23 influenza cases, including 2 infections in one case, who had at least four-fold rising of titers, were analyzed. The antibody titers to influenza viruses in acute blood samples are shown in Table 2. Among 23 acute blood samples from 22 patients, all had antibody titers ranging from < 10 to 20, corresponding to its type/subtype. The antibody titers of 40-80 to A (H3N2) in A (H1N1) cases and to A (H1N1) in A (H3N2) cases could be detected in 3 of 5 cases and 2 of 12 cases, respectively.

Protection rate of influenza vaccine

Table 1 shows the incidence of influenza cases in the vaccine and placebo groups. The incidence among vacciated and placebo subjects was 5/61 (8.2%) and 17/62 (27.4%), respectively. The 8.2% of influenza cases found in vaccine group was significantly lower than 27.4% of that in the placebo group (Chi-square test, p = 0.01). The protection rate of the influenza vaccine was 71%.

Of 61 patients in the vaccine group, 4 of A (H3N2) and 2 of A (H1N1) virus infections occurred in 5 cases (Table 3). All of them were non-responders to the vaccination, showing no increase in HI antibody titers. Even though 2 influenza infections were shown in one case, the first infection with A (H3N2) appeared at only day 8 and the second infection with A (H1N1) occurred at 11 months after vaccination. Of the vaccine group, 5 influenza cases occurred at 7, 7, 10, 11 and 11 months after vaccination.

Table 1. Occurrence of influenza in COPD patients with acute respiratory illness within 1-year after influenza vaccination

Type/subtype	No. of positive for influenza			P-value
	Total (n = 123)	Vaccine (n = 61)	Placebo (n = 62)	
Influenza A				
A(H1N1)	5 (22.8)	2 (40)	3 (17.6)	
A(H3N2)	11 (50.0)	3 (60)	8 (47.1)	
A	3 (13.6)	-	3 (17.6)	
Influenza B	1 (4.5)	-	1 (5.9)	
Untypeable**	2 (9.1)	-	2 (11.8)	
Total	22 (17.9%)	5 (8.2%)	17 (27.4)	0.01*

* Significant difference between vaccine and placebo groups at level p-value < 0.05

** Demonstration of a 4-fold or greater increase in antibody titer of both influenza A and B resulted either influenza A or influenza A&B

Table 2. Detection of antibody titers in 23 acute blood samples from 22 patients

Infected Type (subtype)	Antibody to	No. of acute blood samples at HI antibody titer of				
		< 10	10	20	40	80
A(H1N1)	A(H1N1)	-	3	2	-	-
(n = 5)	A(H3N2)	-	-	2	2	1
	B	3	2	-	-	-
A(H3N2)	A(H1N1)	7	2	1	1	1
(n = 12)	A(H3N2)	6	2	4	-	-
	B	11	-	1	-	-
A	A(H1N1)	-	2	1	-	-
(n = 3)	A(H3N2)	1	2	-	-	-
	B	3	-	-	-	-
B	A(H1N1)	-	-	1	-	-
(n = 1)	A(H3N2)	-	-	-	1	-
	B	1	-	-	-	-
Untypeable	A(H1N1)	1	1	-	-	-
(n = 2)	A(H3N2)	2	-	-	-	-
	B	2	-	-	-	-

Table 3. Occurrence of influenza in vaccine group after vaccination

No.	Time after Vaccination (months)	HI antibody titer in				Infected with Type (subtype)
		B1	B2	Ba	Bc	
1	7	< 10	< 10	< 10	40	A(H3N2)
2	10	160	80	20	160	A(H3N2)
3	7	20	20	20	80	A(H3N2)
4	11	20	20	10	≥ 640	A(H1N1)
5	8 days	< 10	NA	40	320	A(H3N2)
	11	< 10	20	10	320	A(H1N1)

Note: B1, baseline before vaccination
B2, one-month after first dose receiving
Ba, acute serum of ARI
Bc, convalescent serum of ARI
NA, not available

Prevalence of HI antibodies to influenza viruses

The results of HI antibodies to influenza viruses in serum from 123 patients are shown in Table 4. The seropositivity rates of influenza virus infections in vaccine and placebo groups were not

Table 4. Prevalence of HI antibodies to influenza viruses in 123 patients with COPD

Type (subtype)	No. (%) seropositive at HI antibody titers of	
	≥ 10	≥ 40
Influenza A		
A(H1N1)	62 (50.4)	27 (21.9)
A(H3N2)	67 (54.5)	35 (28.5)
Influenza B	22 (17.9)	5 (4.1)

different. Of 123 patients, HI antibodies to influenza A (H1N1), A (H3N2) and B viruses were detected in 62 (50.4%), 67 (54.5%) and 22 (17.9%), respectively. However, the patients who had protective antibody titers of ≥ 40 to A(H1N1), A(H3N2) and B viruses were 21.9%, 28.5% and 4.1%, respectively.

Discussion

Influenza viruses are a major cause of mortality and serious morbidity in the elderly, particularly patients with COPD. Most patients in this study were elderly, therefore, all of them were at high risk for contracting influenza. Recommendations for the use of influenza vaccines have been given in several countries for influenza prevention in these groups[3].

An attempt to investigate the cycle of influenza control in the present study, the prevalence, occurrence, seroprotective titer of influenza infection in both vaccine and placebo groups were determined. The occurrence of influenza in the placebo group was found to be 27.4%. This was not different from the general occurrence rate throughout the country[7] and in patients with chronic bronchitis in previous studies[1,8-10]. The infection rate in this study was also not different from the 20% infection rate found in patients with chronic airflow limitation over the age of 50 years[11] and in individuals aged 45-64 years old[12].

This study found that influenza cases of 8.2% in the vaccine group was significantly lower than that of 27.4% in the placebo group. The data here revealed that the influenza vaccination in COPD patients was effective in reducing influenza cases. The 71% protection rate of the vaccine aginst influenza in the present study was higher than in previous studies[13,14].

The 12 laboratory-confirmed influenza subtypes in the placebo group were found to be the following: influenza A (H3N2), A (H1N1) and B were 8 (66.7%), 3 (25.0%) and 1 (8.3%), respectively. These findings indicated that during 1997-1998, influenza A (H3N2) was the most prevalent influenza virus subtype, whereas influenza A (H1N1) and B were much less prevalent. Influenza A (H1N1) infection in the present study was detected in 25.0% of cases which was higher than 2-16% of cases previously reported[7]. The higher difference of influenza A (H1N1) might result from the reduction of homologous antigens. The circulating influenza A (H1N1) in 1997 and 1998 were A/Beijing/262/95 and A/Johannesburg/82/96, and A/Wuhan/371/95, respectively[7], while the vaccine strain of A (H1N1) in the present study was A/Texas/36/91. Even though there was a difference of antigens between the vaccine strains and isolates, the vaccine could still be used for protection against influenza. Previous reports have demonstrated that the protection rates depended on the antigenic homology; 100%, 50% and 9% homology provided protection rates of 88%, 69% and 38%, respectively[15,16].

All 23 laboratory-diagnosed cases by serology showed antibody titers of < 10 to 20 corresponding to its type/subtype in acute serum samples. These results confirmed that the protective HI antibody titers were ≥ 40. Similar results were found in previous studies of HI antibody titers of approximately 1:30-1:40 which represent a 50% protective level of antibodies[17,18]. The relationship between HI antibody and the incidence of clinical influenza has demonstrated that the attack rates showed a sharp drop-off in individuals with titers of 16 to 32 and were negligible in individuals with titers of ≥ 64[19]. It is well known that serum IgG antibody against H plays a major role in protection against influenza[20,21]. Several studies have shown that susceptibility to influenza virus infection is inversely related to titer of serum IgG HI antibody[18-20]. In addition, the presence of serum HI antibody is reported to reduce the severity of infection[22,23] and to decrease virus shedding[24,25].

Although several studies of seroepidemiology have demonstrated that influenza viruses are prevalent in the elderly, the immune status with protective HI titers of ≥ 40 to influenza viruses is not clear. In humans, HI antibody is first detectable 4-7 days after infection, reaches peak titers at approximately 14-21 days[26] and persists for years[27]. The antibody persistence depends on the time of exposure, prime-boosted exposure to virus antigens and the effect of age on the humoral immune response. The seroprevalence of influenza viruses A (H1N1), A (H3N2) and B were 50.4%, 54.5% and 17, 9%, respectively. However, findings in the present study indicated that an HI antibody titer of 40 was the protective level.

The COPD patients who had protective antibody titers of A (H1N1), A (H3N2) and B were 21.9%, 28.5% and 4.1% respectively. Therefore, the patients in the present study without protective HI antibodies to A (H1N1), A (H3N2) and B viruses were 78.1%, 71.5% and 95.9%; such patients are considered to be at high risk for contracting influenza and recommended to have vaccination[28,29]. In addition, the present study also found evidence of the first influenza cases in non-responders, which occurred at 7 months after vaccination, might support annual immunization.

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การป่วยเป็นโรคไข้หวัดใหญ่ และระดับป้องกันการติดเชื้อไข้หวัดใหญ่โดยวิธีซีโรโลยี่ในผู้ป่วยโรคปอดอุดกั้นเรื้อรังในการศึกษาวัคซีน

อุไรวรรณ โฆษิตานนท์, ระวีวรรณ ขันหยก, จันทพงษ์ วะสี, พูนทรัพย์ วงค์สุรเกียรติ, ทัศนียา สุธรรมสมัย, นันทา มาระเนตร์

วัตถุประสงค์ *: เพื่อสืบสวนหาความชุก, การป่วยเป็นไข้หวัดใหญ่ และระดับป้องกันการติดเชื้อไข้หวัดใหญ่โดยวิธีซีโรโลยี่ในผู้ป่วยโรคปอดอุดกั้นเรื้อรังในช่วงหนึ่งปีของการศึกษาวัคซีนไข้หวัดใหญ่*

วัสดุและวิธีการ *: ศึกษาผู้ป่วยโรคปอดอุดกั้นเรื้อรัง จำนวน 123 รายช่วงระหว่างปี พ.ศ. 2540 ถึง 2541 อายุของผู้ป่วย 61 รายในกลุ่มวัคซีน และ 62 รายในกลุ่มควบคุม คือ 67.6±8.0 และ 69.1±7.5 ปี ตามลำดับ วัคซีนประกอบด้วยเชื้อไวรัสไข้หวัดใหญ่สายพันธุ์ A/Texas/36/91 (H1N1), A/Nanchang/933/95 (H3N2) และ B/Harbin/07/94 ตรวจหาแอนติบอดีย์ต่อเชื้อไข้หวัดใหญ่โดยวิธีฮีแมกกลูติเนชั่น อินฮิบิชั่น ซึ่งมีสายพันธุ์ของวัคซีนเป็นแอนติเจน*

ผลการศึกษา *: เหตุการณ์ไข้หวัดใหญ่ซึ่งตรวจโดยวิธีทางซีโรโลยี่เกิดขึ้นเป็นจำนวน 22 ใน 123 ราย (ร้อยละ 17.9) ใน 17/62 (ร้อยละ 27.4) รายของกลุ่มควบคุมซึ่งใช้เป็นตัวแทนของการติดเชื้อตามธรรมชาติ พบไข้หวัดใหญ่สายพันธุ์ A (H1N1), A (H3N2), ทัยป์เอ, ทัยป์บี และจำแนกไม่ได้ ดังนี้ 3 (ร้อยละ 17.6), 8 (ร้อยละ 17.1) 3 (ร้อยละ 17.6), 1 (ร้อยละ 5.9) และ 2 (ร้อยละ 11.8) ราย ตามลำดับ พบผู้ป่วยไข้หวัดใหญ่, ร้อยละ 8.2 ในกลุ่มวัคซีนน้อยกว่าร้อยละ 27.4 ในกลุ่มควบคุมอย่างมีนับสำคัญทางสถิติ (ไค-สแคว์ร์, p = 0.01) อัตราการป้องกันการติดเชื้อของวัคซีนไข้หวัดใหญ่คือร้อยละ 71 ตรวจหาแอนติบอดีย์ไตเตอร์ในตัวอย่างตรวจเลือดเมื่อเริ่มป่วย จำนวน 23 ตัวอย่างจากผู้ป่วยจำนวน 22 ราย พบว่าทุกตัวอย่างมีไตเตอร์อยู่ระหว่าง < 10 ถึง 20 ผู้ป่วย 5 ราย ในกลุ่มวัคซีนตรวจพบการติดเชื้อไข้หวัดใหญ่ในเวลา 7 เดือน 2 ราย, 10 เดือน 1 ราย และ 11 เดือน 2 ราย หลังจากได้รับวัคซีน แอนติบอดีย์ไตเตอร์ระดับ ≥ 10 vs ≥ 40 ต่อเชื้อไข้หวัดใหญ่สายพันธุ์ A (H1N1), A (H3N2) และ B ดังนี้ ร้อยละ 50.4 vs 21.9, 54.5 vs 28.5 และ 17.9 vs 4.1 ตามลำดับ*

สรุป *: การศึกษานี้ชี้ให้เห็นว่าในช่วงปี พ.ศ. 2540-2541 ผู้ป่วยไข้หวัดใหญ่ที่ติดเชื้อตามธรรมชาติพบร้อยละ 27.4 เชื้อไข้หวัดใหญ่สายพันธุ์ A (H3N2) มีความชุกมากกว่า A (H1N1) และทัยป์บี วัคซีนไข้หวัดใหญ่ที่ศึกษาได้ผลเป็นประโยชน์อย่างแท้จริง ระดับแอนติบอดีย์ไตเตอร์ ≥ 40 เป็นระดับป้องกันการติดเชื้อ ผู้ป่วยซึ่งไม่มีแอนติบอดีย์ระดับป้องกันต่อสายพันธุ์ A (H1N1), A (H3N2) และทัยป์บี คือร้อยละ 78.1, 71.5 และ 95.9 จัดเป็นกลุ่มเสี่ยงสูงต่อไข้หวัดใหญ่*