Evaluation of clinical data and antibody response following influenza vaccination in patients with chronic obstructive pulmonary disease

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SUMMARY

The present study investigated the antibody response against influenza vaccine and also the efficacy of vaccination on clinical findings in patients with Chronic Obstructive Pulmonary Disease (COPD) following influenza vaccination. A total of 82 cases with COPD (44 cases as vaccinated and 38 cases as unvaccinated) were evaluated clinically and 21 healthy volunteers were also included in the study as a control group. Influenza (A and B) Ig M and Ig G parameters were analyzed quantitatively in blood samples of the vaccinated group and healthy volunteers by ELISA method once before vaccination and one month and one year after vaccination. The presence of dyspnoea, increased sputum production and/or purulence were accepted as criteria of acute exacerbation. The number of hospital presentations was significantly lower in the vaccinated group and higher in severe cases with COPD in unvaccinated group. Vaccinated cases in the study group experienced significantly fewer episodes of pneumonia, hospitalization and intensive care. Quantitative influenza (A and B) antibody IgG levels significantly increased in these patients as well. In conclusion, seasonal influenza vaccination with the trivalent influenza split virion vaccine especially in severe or very severe COPD patients who need hospitalization was evaluated as beneficial in clinical use.

KEY WORDS: COPD, Influenza, Vaccine, Antibody response, Clinical evaluation

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) has become a leading cause of morbidity and mortality all over the world. It ranks fourth as cause of death in the world. It is expected to rank third as a consequence of the increase in smoking rates in 2020 (Murray *et al*., 1997). Acute exacerbation, which is considered the worsening of the previous stabile status of the disease is seen approximately 1-4 times a year.

Exacerbation of the disease is frequently attributable to infections. Twenty-five to thirty percent of infections triggering COPD exacerbations are caused by viruses and also bacteria (Donner, 1999). Particularly, influenza virus and *Streptococcus pneumoniae* infections accelerate the development of pneumonia and secondary bacteria and thus increase mortality and morbidity by causing frequent exacerbation in such patients.

For this reason, influenza and pneumococcal vaccinations are recommended for the national and international diagnoses and treatments of the COPD cases (Turkish Thoracic Society COPD Study Group, 2000; British Thoracic Society, 2001; WHO Workshop Report, 2003).

Vaccination of patients with chronic airway diseases such as COPD and the other risk groups increases their life quality and decreases their hospitalization frequency and infection episodes, as well as their treatment costs.

In this study, assessment of antibody response against influenza vaccine and the clinical impact of the vaccination were investigated. The clinical and social benefit of the vaccination on the cas-

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es which required serious and intensive care and/or hospitalization were analyzed on the basis of the antibody levels and clinical data.

MATERIALS AND METHOD

Study population

This prospective study included 82 cases which were hospitalized in Dr. Suat Seren Training and Research Hospital for Chest Diseases and Chest Surgery, with a diagnosis of COPD according to the GOLD (Global Initiative for Chronic Obstructive Lung Disease) criteria between September 2006 and November 2007. The patients with COPD were selected according to the criteria:

1) a forced expiratory volume in one second (FEV1) of <80% of the forced vital capacity (FVC),
2) <200 ml and 12% acute increase in FEV1 or FVC after an inhaled bronchodilator.

Patients with allergy to eggs or patients with associated malignancy were excluded. Ethics committee approval was taken along with written informed consent from all the patients.

Sample collection and vaccination

Blood samples were obtained prior to the administration of the influenza vaccine.

The patients were administered trivalent influenza split virion vaccine (the vaccine included A/Shangdong/9/93 (H3N2), A/Texas/36/91 (H1N1) and B/Panama/45/90. Thus the individual groups whether receiving the vaccine or not were named as the "vaccinated group" or "unvaccinated group". Whole blood samples were obtained from 44 vaccinated patients within the 1st and 12th months following administration. Additionally, blood samples were collected from 38 unvaccinated patients with COPD and 21 healthy volunteers in order to analyze influenza (A and B) Ig M and Ig G levels. The subgroups used in the analysis were indicated as naïve/not infected (influenza Ig M and Ig G negative), acute infection (influenza Ig M positive and Ig G positive/negative) or previously infected (not infected in the initial stage of the study period) (Ig M negative and Ig G positive). The number of selected individuals in study (vaccinated and unvaccinated) and healthy volunteers has been shown in Table 1. The mean antibody levels in association with each subgroup were also added as subinformation under Table 1.

TABLE 1 - *The number of selected individuals in vaccinated and unvaccinated groups and healthy volunteers.*

Subgroups[1]	*Study Group*[2] *(N=82)*				*Healthy volunteers*[2] *(N=21)*	
	Vaccinated (N=44)		*Unvaccinated N=38)*			
	Inf A	*Inf B*	*Inf A*	*Inf B*	*Inf A*	*Inf B*
naive/not infected	22	28	20	24	4	5
previously infected	20	15	12	12	14	15
acute infection	2	1	6	2	3	1

[1]Subgroups are defined as naïve / not infected (Ig M and Ig G both are negative), previously infected (not infected in the initial stage of study period; Ig M negative and Ig G positive) and acute infection (Ig M positive and Ig G negative /positive). [2]Mean antibody levels in association with each subgroup are as follows: Ig M values (U/mL) for Influenza A in vaccinated, unvaccinated groups and healthy volunteers: Vaccinated Group: 1.2±0.8 (naïve/not infected), 0.0±0.0 (previously infected), 48.9±27.25 (acute infection); Unvaccinated Group: 0.0±0.0 (naïve/not infected), 0.0±0.0 (previously infected), 50.4±22.8 (acute infection); Healthy volunteers: 3.4±2.3 (naïve / not infected), 0.0±0.0 (previously infected), 54.2±18.9 (acute infection). Ig M values (U/mL) for Influenza B in vaccinated, unvaccinated groups and healthy volunteers: Vaccinated group: 3.0±1.9 (naïve/not infected), 0.0±0.0 (previously infected), 24.6±0.0 (acute infection); Unvaccinated Group: 0.0±0.0, 2.9±0.8, 15.6±8.4; Healthy volunteers: 3.2±4.5 (naïve/not infected), 2.8±1.4 (previously infected), 28.9±0.0 (acute infection). Ig G values (U/mL) for Influenza A in vaccinated, unvaccinated groups and healthy volunteers: Vaccinated Group: 0.0±0.0 (naïve/not infected), 63.5±47.9 (previously infected), 27.6±18.4 (acute infection); Unvaccinated Group: 0.0±0.0 (naïve/not infected), 65.5±45.9 (previously infected), 44.8±32.9 (acute infection); Healthy volunteers: 0.0±0.0 (naïve/not infected), 77.8±25.7 (previously infected), 38.2±16.9 (acute infection). Ig G values (U/mL) for Influenza B in vaccinated, unvaccinated groups and healthy volunteers: Vaccinated Group: 2.0±1.1 (naïve/not infected), 41.4±42.8 (previously infected), 0.0±0.0 (acute infection); Unvaccinated Group: 0.0±0.0 (naïve/not infected), 38,9±25,9 (previously infected), 0.0±0.0 (acute infection); Healthy volunteers: 0.0±0.0 (naïve/not infected), 79,1±51,5 (previously infected), 40.7±0.0 (acute infection). Abbreviation; Inf: influenza.

Clinical criteria for COPD patients

All the patients were stratified on the basis of their FEV1 as mild COPD: FEV1 >80% predicted; moderate COPD: FEV1 = 50-79% predicted; severe COPD: FEV1 = 30-50% predicted and very severe COPD: FEV1 = <30% predicted. Data regarding comorbid diseases, history of smoking and treatment history were collected from all patients studied.

A standard treatment regimen was applied according to the GOLD guidelines for the management of COPD. All the factors (drug modulation, checking of inhaler technique, life style modification and exercise) were kept similar between vaccinated and unvaccinated patients in the study period.

The patients who had not smoked for a period of one year were accepted to be ex-smokers. Selection criteria for all cases in the study group was the diagnosis of COPD in the sequential order of submission to the hospital during the clinical evaluation period. All subjects with COPD were evaluated and classified according to the GOLD criteria. In the vaccinated group, 6 mild, 12 moderate, 15 severe and 11 very severe COPD cases were included in the study, whereas 2, 5, 16 and 15 cases regarding mild, moderate, severe and very severe cases in the unvaccinated group, respectively.

Pneumococcal vaccine was not administered to any of the subjects in the study and control groups. Healthy volunteers were selected randomly from the health-care workers or subjects had diseases other than influenza-like illness or pneumonia. Chest-X-ray and laboratory findings were found as normal for all healthy control subjects. Among healthy volunteers, the age interval was 35-70 (mean 62.9±1.5) and none of the healthy individuals selected for the study had a systemic failure (e.g. diabetes, hypertension, congestive heart failure) or an acute and/or a chronic infection (e.g. upper or lower respiratory infection).

The patients who were followed at two month intervals for one year were assessed in terms of hospitalization due to acute exacerbation. Criteria of acute exacerbation were diagnosed and categorized on the basis of clinical criteria of increasing shortness of breath, increase in amount or purulence of sputum. At the same time, the patients were questioned in terms of symptoms of cough, stertorous respiration, nasal flow, fever, malaise and sore throat during each follow up, in addition to the above-mentioned acute exacerbation criteria.

The patients who had infiltration on the posterior-anterior pulmonary graph in addition to leucocytosis and met the acute exacerbation criteria were considered as pneumonia.

The first month when the patient applied to the hospital due to acute exacerbation and the number of exacerbations were recorded in one year follow up.

Duration of the hospitalization due to acute exacerbation was indicated for each group. Furthermore, the patients who underwent intensive care treatment were recorded. The clinical data was collected and documented by using a standard form for each patient.

Analysis of influenza antibodies in the COPD patients

Blood samples were obtained from the patients under appropriate conditions through thin tubes and sent directly to the microbiology laboratory. All blood samples were barcoded after clotting and centrifuged at 2000 g for 10 minutes. Sera from the patients were stored at -20°C till the time of analysis. Samples were analyzed by using microELISA kits (IBL, Immunobiological Laboratories, GmbH, Hamburg, Germany) on automated ETI-Max 3000 microplate analyzer (DiaSorin S.p.A., Saluggia (Vercelli), Italy) in accordance with the recommendations of the manufacturers in terms of Ig M and Ig G for both Influenza A and B, seperately. Four calibrators between 0-150 U/mL were used (standard A: negative control, standard B: cut-off control, standard C: weak positive control, standard D: positive control). Cut-off value was accepted as calibrator B (10 U/mL ±20%).

Accordingly, each sample was considered as negative (<8 U/mL) or positive (>12 U/mL) or within the doubtful interval (grey zone) (8-12 U/mL). The most accurate value for each sample was calculated by using an automated and computer-based program, quantitatively.

The obtained optical density (OD) of the standards (y-axis, linear) were plotted against their concentration (x-axis, logarithmic) using the automated method. A good fit was provided with 4 Parameter Logistics. The numerical data obtained

was classified as <12 U/mL (unresponsive), 12-30 U/mL (low response), 30-100 U/mL (moderate response) and >100 U/mL (high response).

Quality control and validation criteria

The test results were accepted as valid only if the test had been performed by following the manufacturer's instructions. Moreover, the rules of GLP (Good Laboratory Practice) or other applicable standards/laws were strictly applied. All standards were found within the acceptable ranges as stated on the Quality Control Certificate. Final release results of acceptable optical density (OD) ranges for calibrators A, B, C and D were ≤0.150, ≥0.200, ≥0.450 and ≥1.000, respectively. If the criteria were not met, the run was not accepted and repeated.

According to the data supplied by the manufacturer, no interferences had been detected to bilirubin up to 0.3 mg/mL, hemoglobin up to 8.0 mg/mL and triglycerides up to 5.0 mg/mL for all parameters. No cross-reactivity had been found to RSV, Adenovirus and/or Parainfluenza 1/2/3 for Influenza A Ig G and Ig M, and to Bordetella, Gliadin, Helicobacter, transglutaminase for Influenza A Ig A. The performance evaluation of these test systems had been carried out by the manufacturer in comparison with the reference method. Overall sensitivity and specificity for Influenza (A and B) Ig A, Ig M and Ig G were reported between 85% and 100% (Immunobiological Laboratories, 2008).

Statistical analysis

Statistical analysis was conducted by using SPSS 15.0.1 computer program. Distribution of data was evaluated with Kolmogorov-Simirnov test. The data that did not fit the normal dispersion were assessed with Wilcoxon and Mann-Whitney test, while the data which fitted to the normal dispersion was assessed with One Way ANOVA and Paired Sample t test.

Ordinal variables were evaluated with chi-square test. Chi-squared and t- tests were used to compare groups for discrete and continuous variables respectively. The incidence of acute exacerbation was calculated and compared using an incidence density ratio [the ratio of the number of episodes of acute exacerbation over the number and time of follow up of patients (person- years)], estimated by a Poisson model and then calculating the relative risk (RR) and efficacy of the vaccination was calculated with the formula of [(1-RR)*100]. The p-value of 0.05 was considered as the limit of significance.

RESULTS

Clinical data of the patients

Mean ages of the vaccinated and unvaccinated groups were 62.9±1.2 (40-77) and 62.9±1.5 (35-80), respectively. When the groups were assessed in terms of age, sex, systemic additional disease (e.g. hypertension, coronary arterial disease), smoking habit, stage of COPD and survival, there was no statistically significant difference between the groups (p>0.05).

Clinical evaluation of vaccinated and unvaccinated patients with COPD

The evaluation criteria for statistical analysis to calculate the clinical efficacy of influenza vaccination in COPD patients were the frequency of presentation to the hospital due to acute exacerbation, length of hospitalization, infiltration in chest X-ray, presence of pneumonia, requirement of intensive care, the stage of COPD in vaccinated and unvaccinated cases.

The frequency of presentations to the hospital due to acute exacerbation differed between 1 and 4 times (0.8±1.2) in the vaccinated group, whereas between 1 and 5 (1.6±1.3) times in the unvaccinated group in one year follow up. Thus the frequencies in the vaccinated group were 1, 2, 3 and 4 times for 7, 4, 4 and 2 patients, whereas; 1, 2, 3, 4 and 5 times for 9, 9, 8, 2 and 1 patient in the unvaccinated group, respectively.

However, a total of 17 patients in the vaccinated group and 29 patients in the unvaccinated group presented to the hospital again at least once due to acute exacerbation within the year, which was statistically significant (p=0.001). The difference between the duration of hospitalization periods in vaccinated and unvaccinated patients was statistically significant [i.e. hospitalization periods were 7.7±13 days and 17.5±19.5 days for vaccinated and unvaccinated groups, respectively] (p<0.05).

Infiltration in chest X-ray associated with pneumonia was found in 43.5% and 56.5% of the patients in the vaccinated and unvaccinated groups,

TABLE 2 - *Distribution of clinical evaluation criteria for efficacy of influenza vaccination among vaccinated and unvaccinated patients.*

Clinical Evaluation Criteria	*Vaccinated Group N=44 (%)*	*Unvaccinated Group N=38 (%)*	**P*
Application due to AE applied to the hospital (n=46) not applied (n=36)	 17 (38.6) 27 (61.4)	 29 (76.3) 9 (23.7)	 p<0.05, RR=0.38 p<0.05, RR=0.76
Pneumonia occurred (n=24) not occurred (n=59)	 11 (25) 33 (75)	 13 (34.2) 25 (65.8)	p<0.05
IC required (n=19) not required (n=54)	7 (15.9) 37 (84.1)	12 (31.6) 17 (44.7)	p<0.05
Number of acute attack in one year	0.8±1.2	1.6±1.3	p<0.05
Length of hospitalization (day)	7.7±13	17.5±19.5	p<0.05

AE: acute exacerbation, IC: intensive care, RR: relative risk *Chi- squared test.

respectively. Comparison of the groups in terms of occurrence of pneumonia and requirement of intensive care showed that the number of patients in the vaccinated group was less than the patients in the unvaccinated group (p=0.007 and p=0.003 for occurrence of pneumonia and requirement of intensive care, respectively). Distribution of clinical evaluation criteria for efficacy of influenza vaccination among vaccinated and unvaccinated patients has been shown in Table 2.

Among 82 cases in total, 46 cases (17 vaccinated and 29 unvaccinated) were hospitalized, 19 cases were required intensive care and 24 cases had pneumonia. Among hospitalized patients, one with mild and two cases with moderate COPD were present in the vaccinated group. No cases with mild or moderate COPD in the unvaccinated group needed intensive care.

In vaccinated group, two cases with mild or moderate COPD had pneumonia, whereas no cases with mild and one case with moderate COPD suffered from pneumonia in unvaccinated group. Thus, in stages of mild or moderate COPD, there was no statistical difference between the number of patients in case of hospitalization, intensive care and acquisition of pneumonia. All patients treated in the intensive care were evaluated as severe or very severe COPD.

In vaccinated and unvaccinated groups, a total of 19 patients required intensive care in the severe or very severe stage [7 vaccinated and 12 unvaccinated, (p=0.048)]. The rate of vaccinated patients with severe or very severe COPD who were hospitalized were significantly less than the rate of unvaccinated cases [n=6 (severe) and n=8 (very severe) in vaccinated group; n=14 (severe) and n=15 (very severe) in unvaccinated group; p=0.009 and p=0.029 for severe and very severe stages, respectively].

Pneumonia as a complication of influenza was diagnosed as frequently in unvaccinated patients with very severe COPD clinically [n=5 (severe) and n=4 (very severe) as in the vaccinated group; n=4 (severe) and n=8 (very severe) in unvaccinated group; (p=0.015)].

Influenza Ig M and Ig G antibody levels of vaccinated patients and healthy volunteers

When the influenza A and B lg G levels before vaccination of the 65 COPD cases were compared with the influenza Ig G levels in the 21 healthy cases of control group, only influenza B lg G levels were statistically higher in the healthy cases (p=0.001).

When the influenza (A and B) lg G levels of 44 vaccinated cases were measured one month and one year after the vaccination, the values of one month and one year after vaccination were found to be increased significantly (p<0.05) as shown in Figure 1.

After influenza A and B lg M positive patients were excluded (as the increase in the levels of Ig

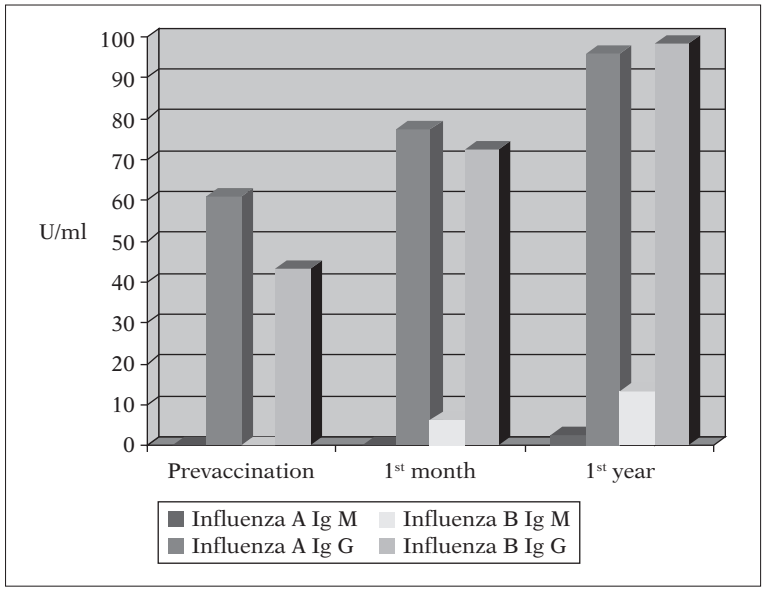

FIGURE 1 - *P values for Influenza A Ig G (prevaccination versus 1st month) = 0.029, Influenza A Ig G (1st month versus 1st year) = 0.021, Influenza A Ig G (prevaccination versus 1st year) = 0.000, Influenza B Ig G (prevaccination versus 1st month) = 0.003, Influenza B Ig G (1st month versus 1st year) = 0.009, Influenza B Ig G (prevaccination versus 1st year) = 0.000.*

M and Ig G might be attributable to acute infection), 22 cases out of 44 cases in the vaccinated group displayed statistically significant increase in the values of influenza A and B Ig G levels after one month and one year following influenza vaccination ($p<0.05$) as shown in Figure 2.

DISCUSSION

The incidence of diagnosed COPD has increased by >40% since 1942 and the illness now serves as the fourth leading cause of death in the world (Niederman, 1997). Over the prolonged chronic course of the disease, episodes of acute exacerbation often occur, each accelerating further decline in lung function.

These episodes have a deleterious effect on patients' quality of life and necessitate utilization of healthcare services including hospitalization and even mechanical ventilation sometimes (Seneff *et al.*, 1995). Generally no more than half of all exacerbations are of bacterial origin, the remainder being due to viral infection of which influenza plays a substantial role (Niederman, 1997). Influenza viruses are a major cause of mortality and serious morbidity in the elderly individuals, particularly in patients with COPD

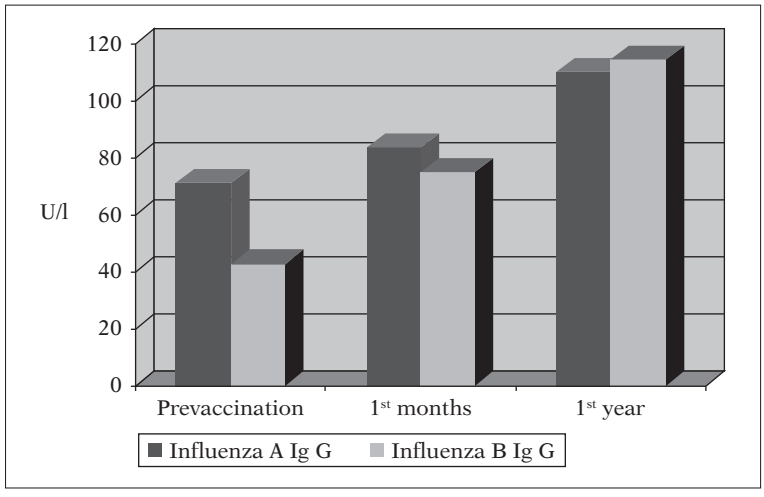

FIGURE 2 - *P values for Influenza A Ig G (prevaccination versus 1st month) = 0.188, Influenza A Ig G (1st month versus 1st year) = 0.001, Influenza A Ig G (prevaccination versus 1st year) = 0.002, Influenza B Ig G (prevaccination versus 1st month) = 0.044, Influenza B Ig G (1st month versus 1st year) = 0.005, Influenza B Ig G (prevaccination versus 1st year) = 0.000.*

(Kositanont *et al*., 2004). Influenza vaccine has proved to be effective in preventing hospital admissions and deaths in populations of elderly people (Nichol *et al.*, 1994; Wiselka, 1994; Rothbarth *et al*., 1995; Elden *et al.*, 2001). Although influenza is not really related to excess mortality in young COPD patients, it can play an important role in causing exacerbations, especially in epidemic seasons (Rothbarth *et al*., 1995).

In the present study, 76.3% and 38.6% of the cases in the unvaccinated and vaccinated groups presented to hospital due to acute exacerbation, respectively.

Smoking, low body temperature, depression, use of psychotrophic medication, poor living conditions, insufficient pulmonary rehabilitation and non-administration of influenza and pneumococcal vaccine have been reported as the causes of repeated hospital presentations (Cao *et al.*, 2006). Lower numbers of exacerbations and influenza infections during follow-up period have also been reported in vaccinated COPD cases in previous studies (Wonngsurakiat *et al*., 2004, Tasbakan *et al*., 2007, Menon *et al*., 2008).

Influenza vaccine has been very effective in protecting the cases against influenza associated acute respiratory diseases, independent from the severity of the airway obstruction. In addition, administration of influenza vaccine is cost effective in terms of protection against exacerbations that would require mechanical ventilation in COPD cases having severe airway obstruction (Wonngsurakiat *et al*., 2003).

The present study found that the vaccinated cases required shorter hospitalization periods, fewer applications and intensive care than the unvaccinated cases in the severe or very severe COPD stage. Additionally, fewer vaccinated COPD cases with pneumonia were diagnosed in comparison with the unvaccinated group.

Administration of influenza vaccine decreases the incidence of pneumonia and can be effective in the management of clinical complications and treatment costs in severe or very severe COPD cases. On the other hand, few studies have documented that there is no reduction in incidence rate of influenza associated respiratory morbidity in patients with COPD (Ramadan *et al*., 2001; Hak *et al.*, 2003). In our study, most of those who died in the vaccinated group were in the severe and very severe stages of COPD and the reason for death was continuity of smoking, respiratory failure associated with the severity of the airway obstruction and congenital hearth failure.

The efficacy of influenza vaccination has been documented to range from 32% to 45% in various clinical trials (Nichol *et al*., 1994; Gross *et al.*, 1995; Nichol *et al*., 1999; Wongsurakiat *et al.*, 2004).

The effectiveness of vaccination in prevention of acute exacerbation in COPD patients in this study was 62% [(1-RR)*100]. Each year, the vaccine components are identified by global surveillance and prediction of the strains most likely to be dominant during influenza seasons in the southern and northern hemispheres. One of the reasons the efficacy of the vaccine shows a high variety in the studies could be attributable to the level of formation of protective antibody by the patients following vaccination and the maintenance of this protection is limited within the influenza season for each year. In addition, the naturally occurring influenza virus strain often varies from year to year, and although the vaccine strain frequently matches the epidemic strain, occasionally it does not.

Currently available influenza vaccines are effective only against infecting strains of the virus that have hemagglutinins of similar antigenic characteristics. When the infecting virus has had minor changes in the hemagglutinins (antigenic drift), the vaccine may provide partial protection. However, a major change in the viral hemagglutinins (antigenic shift) results in the lack of vaccine protection.

In recent years, trivalent vaccines include H3N2 and H1N1 subtypes which have been circulating most prevalently since 1978 all over the world (Murphy *et al*., 1985; Belshe *et al*., 1992). The seasonal influenza vaccine contains three influenza viruses; one influenza A (H3N2) virus, one regular seasonal influenza A (H1N1) virus (not the 2009 H1N1 virus), and one influenza B virus. The vaccine strain in pandemic vaccines worldwide is based on the initial isolate of influenza A/California/7/2009 (H1N1)v or a reassortment based on the same isolated strain and a more fast-growing influenza A(H1N1) strain (PR8) called influenza A/California/7/2009 (H1N1)v-like (Johansen *et al*., 2009).

The composition of the seasonal or pandemic vaccines differ significantly in conditions for virus

propagation, antigen preparation, antigen content and whether they are adjuvanted or not. There are two influenza (flu) vaccines. The "flu shot" is an inactivated vaccine (containing killed virus) given with a needle and injected into the muscle. There also is a nasal-spray flu vaccine (sometimes called LAIV for Live Attenuated Influenza Vaccine) that contains weakened live viruses. Influenza vaccines have been manufactured by a wide range of technologies such as egg-derived, whole-virion, recombinant, and live-attenuated vaccines.

Adjuvants are biochemical substances which have been used for many years in many vaccines to reduce the dose of antigen while producing the same and longer-lasting protective response. Since it was first licensed in 1997, a seasonal influenza vaccine containing the MF59 adjuvant has been used with more than 40 million doses distributed.

In current adjuvanted pandemic vaccines the oil-in-water adjuvants (squalene-based), MF 59 and AS03, and the aluminium phosphate adjuvant have allowed reduction of the haemagglutinin content per dose (Demicheli *et al.*, 2004; CDC, 2008; Schultze *et al.*, 2008; Johansen *et al.*, 2009).

In the present study, an inactivated, non-adjuvanted influenza vaccine, trivalent types A and B (Split Virion) containing 3 strains of influenza virus cultivated on embryonated eggs was administered to the COPD cases by intramuscular injection.

This trivalent seasonal vaccine contains thiomersal, a long-used mercury-containing preservative needed to maintain sterility in many vaccines during production.

The seasonal flu vaccine is unlikely to provide protection against 2009 H1N1 influenza because the new H1N1 virus is very different from the seasonal H1N1 virus in the seasonal flu vaccine (Centers for Disease Control and Prevention, 2009; Garten *et al.*, 2009).

However, the seasonal influenza vaccine may help protection against novel pandemic influenza A (H1N1), according to the results of some recent studies in the Mexico population (Laurie, 2009). These results are to be considered cautiously and in no way indicate that seasonal vaccine should replace vaccination against pandemic influenza A/H1N1 2009. The available evidence, although incomplete, suggests that seasonal vaccines will confer little or no protection against influenza A/H1N1. In a letter by Xing and Cardona (Xing and Cardona, 2009), some similar epitopes on nucleoprotein, matrix and haemagglutinin proteins of two influenza strains found in the seasonal influenza vaccine of this year have determined that seasonal influenza vaccination would be effective against pandemic influenza as well. However, further studies are needed to put forward the immunological response and the association of seasonal and pandemic influenza vaccination in various populations and risk groups.

As it is well known, the gold standard for laboratory diagnosis of influenza infection is the isolation of virus by cell culture, subtyping or direct detection of influenza antigen itself.

In some studies (Kositanont *et al.*, 2004; Wongsurakiat *et al.*, 2004), only clinical diagnosis criteria have been used to identify clinical influenza infection.

However, serological measurement of immune response and clinical diagnosis together are useful to overcome the difficulties in the studies measuring vaccine efficacy and clinical evaluation of influenza (Govaert *et al.*, 1994; Nichol *et al.* 1994; Nichol, 1998).

The present study evaluated the efficacy of vaccination on the basis of antibody response and clinical criteria. Although haemagglutination inhibition (HAI) and microneutralization assays are frequently used in most studies and accepted as reference methods in measuring the protective antibody levels against influenza virus, (i.e. the efficacy of vaccination), there have been some disadvantages of these assays reported previously. In these assays, the nonspecific inhibitors affect the sensitivity and neutralization of inhibitors increases the turnover time of the method up to two days.

Therefore, a standardized ELISA is found to be more sensitive, specific and faster than HAI method (Benne *et al.*, 1995; Leinikki *et al.*, 1997). ELISA detects both neutralizing (anti-haemagglutinin and neuroaminidase) and non neutralizing antibodies (against matrix and nucleoprotein antigens) at the same time (Snyder *et al.*, 1988; Jawetz *et al.*, 1989; Leonardi *et al.*, 1994). It is possible to perform ELISA with only one serum dilution and quantification of antibodies. Besides these, in some routine laboratories, it is difficult to apply the preanalytic process such as purifi-

cation of serum from inhibitors, recovery of hen erythrocytes and supply the haemagglutinin antigens. As ELISA is not time-consuming and has relatively a high sensitivity and specificity, a standardized ELISA has been reported as useful for detection of seroconversion in vaccinated individuals and especially suitable for virus laboratories in hospitals.

The present study used a commercially available and validated ELISA kit which was thought to be more suitable, easy and reproducible to apply in our laboratory to obtain more precise results.

A study which has underlined that influenza vaccine maintains its efficacy for a period of one year found high ratios of antibodies and protection. Additionally, only one dosage of the vaccine instead of two dosages would be sufficient for the adults as adequate antibody response ratios have been detected following the administration of the first dosage of the vaccine (Wongsurakiat *et al*., 2004). Another study (Sayan *et al*., 2005) focused on the seroconversion with ELISA on patients administered with influenza virus vaccine reporting that IgG geometrical average titers are higher than IgA and IgM.

The authors thought this was important in terms of protection and it could be detected even after the sixth month.

The antibody responses following vaccination, Ig G antibody responses significantly increase in the first and sixth months for H3N2 and in sixth month for H1N1. Otherwise, Ig G response against influenza B virus decreases within the sixth month of vaccination. On the other hand, some patients have protective antibody levels, though low, prior to vaccination. This indicates that these patients produce cross reactive antibodies against different influenza strains or that they have encountered the same influenza viruses previously.

The present study also found that protective antibody levels (Ig G) of the vaccine remained for one year. Additionally, the levels of the protective influenza A lg G antibody titers existing beforevaccination were higher than those of the influenza B Ig G antibody titers.

The ratio of these protective antibodies increased in one year at a ratio that was statistically significant. This finding together with the clinical data was interpreted as supporting the efficacy of vaccination.

Influenza vaccination efforts should continue to be targeted toward persons, especially in children and adults of any age who are immunosuppressed or have other chronic medical conditions or at increased risk for influenza complications. The immunological response to influenza vaccination in patients who are at especially high risk (e.g. haemodialysis, transplantation, coronary artery disease) have been investigated (Mazzone *et al*., 2001; Keshtkar-Jahromi *et al*., 2009). Humoral immune response to trivalent seasonal influenza vaccine has been sought in different countries, in elderly people, in patients with pulmonary diseases, renal diseases, diabetes mellitus, cancer and haemophilia, and in those with HIV infection (Brydak and Machala, 2000).

Some investigations indicated a poorer humoral response to influenza vaccine in these groups, while others showed responses comparable to those in healthy individuals. It was thought that these differences might be explained by differences in types and stages of the chronic diseases, in the treatment and composition of influenza vaccines, and also patients' ages, vaccination history and prevaccination antibody titres.

In conclusion, seasonal influenza vaccination especially in severe or very severe COPD patients with the trivalent influenza split virion vaccine (A/Shangdong/9/93 (H3N2), A/Texas/36/91 (H1N1), B/Panama/45/90) was evaluated as beneficial in decreasing clinical symptoms (such as exacerbations), medical applications (follow-up procedures, treatment and intensive care), complications of influenza disease (such as pneumonia) and duration of hospitalization.

Seasonal influenza vaccination in COPD patients as a high risk group with a high incidence may improve the clinical benefits and help future efforts to prevent pandemics in which H1N1 seems to be the most widely circulating influenza type among the population worldwide. However, vaccination must be investigated for a newer generation of influenza vaccines with a composition of most recent strains emerging in the future and more efficient and practical methodologies to measure the efficacy of vaccination should be developed in clinical use.

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