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Research Article

Antibody Immune Response to Influenza Virus Vaccine in Asthma and COPD Patients.

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Abstract

Introduction: Asthma and chronic obstructive pulmonary disease (COPD) are obstructive airway diseases with chronic inflammation of the respiratory tract but they differ markedly in the inflammatory cells and mediators involved. Vaccination against influenza virus is recommended in both diseases. To the best of our knowledge there are no published studies comparing the vaccination antibody immune response between asthmatic and COPD patients **Aim of study:** Our study aimed to assess the difference in the antibody immune response to trivalent inactivated influenza virus vaccine among patients with bronchial asthma compared to patients with COPD four weeks after vaccination. **Subjects and Methods:** The study included 40 patients divided into 2 groups; 20 asthmatic patients and 20 COPD patients. Serum anti-Influenza A IgG and anti-Influenza B IgG antibodies levels before and 4 weeks after vaccination in all patients were measured by ELISA. **Results:** The results of the study revealed that IgG antibody responses following inactivated influenza virus vaccine significantly increased after 4 weeks in both groups with no significant difference between patients with bronchial asthma and COPD patients. **Conclusion:** Our data suggests that antibody immune response to seasonal inactivated influenza virus vaccine in asthmatics is comparable with that in COPD patients with no significant difference.

Keywords: Asthma, COPD, Influenza vaccine, Humoral immune response.

Introduction

Asthma is a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, tightness of chest and cough that vary over time and in intensity, with variable expiratory airflow limitation **(GINA, 2014)**.

COPD is a disease state characterized by persistent airflow limitation, usually progressive, not fully reversible, and is associated with an enhanced chronic inflammatory response in the airways and the lungs to inhaled noxious particles or gases. Exacerbations and comorbidities contribute to the overall severity in individual patients **(GOLD, 2014).**

Asthma and chronic obstructive pulmonary disease (COPD) are both obstructive airway diseases that involve chronic inflammation of the respiratory tract, but the type of inflammation differs markedly between both diseases, with different patterns of the inflammatory cells and the mediators involved **(Barnes, 2008)1.**

Bronchial biopsies from asthmatics show infiltration with eosinophils, activated mast cells, and T cells which are predominantly Th2 cells. In bronchial biopsies, small airways, and lung parenchyma from patients with COPD, infiltration of T cells (predominantly Th1 and type 1 cytotoxic T [Tc1] cells) is found together with increased numbers of neutrophils and macrophages **(Barnes, 2008)2.**

A pivotal role in the pathogenesis of asthma is played by the CD4+ lymphocytes with a Th2 cytokine pattern

(Mazzarella et al., 2000). The TH2 cells secrete cytokines that includes interleukin-4 (IL-4), IL-5, IL-9, and IL-13, all of which contribute to the various manifestations of allergic inflammation and disease **(Barrios et al., 2006).**

In COPD, there is increase in T-lymphocytes with a shift in the balance of the CD4/ CD8 +ve T-lymphocyte ratio in favor of the CD8. Indeed, CD8 +ve cytotoxic T- lymphocytes infiltrate the central airways, the peripheral airways and the lung parenchyma, suggesting an inflammatory process along the entire tracheobronchial tree **(Saetta et al., 2001).**

According to **GINA 2014** and **GOLD 2014**, vaccination against influenza virus is recommended in both asthmatic and COPD patients.

According to **Centers for disease control and prevention (CDC);** Influenza is one of the orthomyxovirusfamily. It is single-stranded, helically shaped, RNA virus. Basic antigen types A, B, and C are determined by the nuclear material. Influenza A viruses are divided into subtypes based on two proteins on the surface of the virus: the hemagglutinin (H) and the neuraminidase (N). There are **18** different hemagglutinin subtypes and **11** different neuraminidase subtypes. Influenza A viruses can be further broken down into different strains. Current subtypes of influenza A viruses found in people are influenza A (H1N1) and influenza A (H3N2) viruses.

According to **CDC,** there are three influenza viruses in each injected seasonal influenza vaccine (trivalent inactivated vaccine, TIV): one influenza type A subtype H3N2 virus strain, one influenza type A subtype H1N1 (seasonal) virus strain, and one influenza type B virus strain. Each TIV contains 15 micrograms (μg) of the haemagglutinin HA of each of the recommended strain component**.**

Studies investigating the efficacy and immunogenicity of flu vaccine have been conducted in a variety of settings and populations using several different outcomes, yet **according to the best of our knowledge** there are no previously published studies comparing the antibody immune response following influenza virus vaccine between asthmatic and COPD patients, as these two diseases differ markedly in the patterns of inflammatory cells and mediators involved.

Aim of the study

Our study was designed to assess the difference in the antibody immune response to trivalent inactivated influenza virus vaccine among patients with bronchial asthma in comparison to patients with chronic obstructive pulmonary disease four weeks after vaccination with trivalent inactivated influenza virus vaccine.

Subjects and Methods

Patients

The study was aprospective study conducted on 40 eligible subjects comprised two groups of comparable ages; a group of 20 atopic asthmatic patients and another group of 20 COPD patients. The clinical component of the study was conducted between Septembers to November 2012, and patients were recruited from chest outpatient clinic, faculty of medicine, Zagazig University.

Asthma patients were diagnosed according to **GINA** guidelines 2010. Atopy was defined by the presence of a positive skin prick test reaction to one or more common allergens. COPD patients were diagnosed according to **GOLD** guidelines 2011.

Exclusion criteria included patients known to have anaphylactic hypersensitivity to eggs or to other components of the influenza vaccine, those with history of severe allergic reaction to a previous influenza vaccine, those with history of Guillain-Barré syndrome within 6 weeks following a previous dose of influenza vaccine. Further exclusion criteria included patients with FEV1< 60% of predicted or with current moderate or severe febrile illness or patients associated with diabetes, liver cirrhosis, chronic kidney disease, autoimmune diseases and malignancy. Patients who were receiving drugs which may affect the results of the study as oral steroids and antihistamines were also excluded.

Methods

Trivalent inactivated influenza virus (TIV)

The patients were vaccinated intramuscularly in deltoid with seasonal trivalent inactivated influenza vaccine (**Fluarix** Influenza Vaccine **2012/2013** Manufactured by GlaxoSmithKline Biologicals, Dresden, Germany).

Fluarix vaccine is a Suspension for injection supplied in 0.5-mL single-dose prefilled syringes for intramuscular injection.

Fluarix vaccine is inactivated influenza vaccine (split virion), containing antigens (propagated in embryonated eggs) equivalent to the following types and subtypes:

A/California/7/2009 (H1N1)pdm09-like strain.
A/Victoria/361/2011 (H3N2)-like strain.
B/Wisconsin/1/2010-like strain.

With each vaccine contains 15 microgram of HA antigen of each strain.

Serum anti-influenza A IgG and anti-influenza B IgG

Venous blood samples for antibody assays were obtained from all the participants under appropriate conditions at base line prior to the administration of the influenza vaccine. Another blood samples for antibody assays were collected again from all the participants four weeks later after vaccination. In the present study samples were analyzed by using a commercially available and validated ELISA kits (IBL, Immunobiological Laboratories, GmbH, Hamburg, Germany).

Ethical Consideration

After applying the inclusion and exclusion criteria, all the participants gave an informed consent before any procedures were performed. It included an explanation of the aim and procedures of the study, benefits they would get from flu vaccine, and the possible adverse effects of the vaccine that may occur.The study protocol and consent procedures were reviewed and approved by the ethics committee of Faculty of Medicine- Ain Shams University.

Statistical analysis

All data were collected, tabulated and statistically analyzed using SPSS 15.0 for windows (SPSS Inc., Chicago, IL, USA).

Continuous Quantitative variables e.g. age and FEV1 were expressed as the mean ± SD & range (min-max), and categorical qualitative variables were expressed as an absolute frequencies "number"& relative frequencies (percentage).

Continuous data were checked for normality by using **Kolmogorov-Smirnov test**. **Independent Student T-test** was used to compare two groups of normally distributed data. **Paired T test** was used to compare normally distributed data between two dependent groups (before vs after). Categorized data were compared using the **Chi-square (χ^2) test**. **Pearson's correlation coefficient** was used for correlations of normally distributed variables.

A p-value of less than 0.05 was considered significant.

Results

The study included 40 patients (20 patients with asthma and 20 patients with COPD. There was a significant association between gender & disease as 85% of COPD patients were males vs 50% in asthmatic patients (p=0.018) as shown in ***Table (1).***

Table 1: Comparison between patients with asthma & patients with COPD as regard gender.

Sex		Groups	
		Asthma	COPD
Female	N	10	3
	%	50.00	15.00
Male	N	10	17
	%	50.00	85.00
Chi-square	X^2	5.584	
	P-value	0.018	

There was non-significant difference between patients with asthma and patients with COPD as regard age (Mean age: 46.95 vs 49.65 years, respectively, p=0.223).

Similarly, there was no significant difference between patients with asthma and patients with COPD as regards pre-vaccination IgG A level (U/ml) (Mean: 40.00 vs 44.5, respectively, p=0.362), or as regard pre-vaccination IgG B level (U/ml) (Mean: 35.4 vs 36.150, respectively, p=0.899).

There was non-significant difference between patients with asthma and patients with COPD as regard post-vaccination IgG A level (U/ml) (Mean: 89.5 vs 92.0, respectively, p=0.747).There was also a non-significant difference between patients with asthma and patients with COPD as regard post-vaccination IgG B level (U/ml) (Mean: 85.250 vs 87.750, respectively, p=0.794) as shown in ***Table (2).***

Table 2: Comparison between patients with asthma & patients with COPD as regard post-vaccination IgG A level (U/ml) and as regard post-vaccination IgG B level (U/ml).

Groups	Post-vaccination IgG A level (U/mL)						T-test	
	Range			Mean	±	SD	T	P-value
Asthma	30.000	-	130.000	89.500	±	28.140	-0.326	0.747
COPD	60.000	-	130.000	92.000	±	19.695		
Groups	**Post-vaccination IgG B level (U/mL)**						**T-test**	
	Range			**Mean**	**±**	**SD**	**T**	**P-value**
Asthma	35.000	-	150.000	85.250	±	30.413	-0.262	0.794
COPD	30.000	-	150.000	87.750	±	29.845		

There was a significant increase in IgG A& IgG B level with vaccination in patients with asthma (p<0.001). Mean IgG A level increased from 40.0 U/ml to 89.5 U/ml while Mean IgG B increased from 35.4 U/ml to 85.250 U/ml as shown in ***Table (3).***

Table 3: Change in IgG A& IgG B level (U/ml) with vaccination in patients with asthma

Asthma				Differences		Paired test	
	Mean	±	SD	Mean	SD	T	P-value
PrevaccinationIgG A (U/ml)	40.000	±	16.222	-49.500	16.851	-13.137	0.000
Postvaccination IgG A (U/ml)	89.500	±	28.140				
Prevaccination IgG B (U/ml)	35.400	±	17.031	-49.850	23.214	-9.604	0.000
Postvaccination IgG B (U/ml)	85.250	±	30.413				

There was a significant increase in IgG A& IgG B level with vaccination in patients with COPD ($p<0.001$). Mean IgG A level increased from 44.5 U/ml to 92 U/ml while Mean IgG B increased from 36.150 U/ml to 87.750 U/ml as shown in ***Table (4)*.**

Table 4: Change in IgG A& IgG B level (U/ml) with vaccination in patients with COPD.

COPD				Differences		Paired test	
	Mean	±	SD	Mean	SD	T	P-value
Prevaccination IgG A (U/ml)	44.500	±	14.591	-47.500	16.819	-12.630	0.000
Postvaccination IgG A (U/ml)	92.000	±	19.695				
Prevaccination IgG B (U/ml)	36.150	±	19.885	-51.600	26.301	-8.774	0.000
Postvaccination IgG B (U/ml)	87.750	±	29.845				

Discussion

The results of the present study revealed that the IgG antibody responses following seasonal inactivated influenza virus vaccine significantly increased after 4 weeks in both asthma and COPD groups, yet there was no significant difference in the IgG antibody immune response to vaccination between patients with bronchial asthma and COPD patients.

The assay used for measuring antibody (serological) response to influenza vaccines in humans is an important concern for interpreting those responses. Several assays for HA and NA antibody have been used and have likely contributed to the varied immunogenicity data. These serological tests include the hemagglutination inhibition (HAI) test which is the most widely used assay, Neuraminidase inhibition (NAI) assay, virus neutralization test (neutralization assay), other diagnostic tests such as complement fixation and enzyme-linked immunosorben assay (ELISA) are useful and can be used for serological diagnosis **(WHO, 2011).**

Comparing both haemagglutination inhibition (HAI) assay and ELISA, in measuring the protective antibody levels against influenza virus, (i.e. the efficacy of vaccination), haemagglutination inhibition (HAI) are frequently used in most studies and accepted as reference method, yet 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. ELISA detects both neutralizing (anti-haemagglutinin and neuroaminidase) and non-neutralizing antibodies (against matrix and nucleoprotein antigens) at the same time (**Anar et al., 2010).**

Our present study evaluated the immune response after inactivated influenza virus vaccination on the basis of IgG antibody response. Blood Samples were analyzed for influenza A and B IgG antibodies level by using microELISA kits (IBL, Immunobiological Laboratories, GmbH, Hamburg, Germany).

Studies investigating the efficacy and immunogenicity of flu vaccine have been conducted in a variety of settings and populations using several different outcomes, yet **according to the best of our knowledge** there are no previously published studies comparing the antibody immune response following influenza virus vaccine between asthmatic and COPD patients like our own work, which is thought to be a novel study.

Many studies about humoral and cell-mediated responses to influenza vaccination have been conducted among children and adults..Vaccine-induced protection is considered to be correlated to serum antibodies. Increased levels of antibody induced by vaccination protect against strains that are antigenically similar to those strains of the same type or subtype included in the vaccine. High titers of strain-specific antibody after vaccination are detected in most healthy children and adults **(ACIP, 2013).**

Traditionally, studies of the immunogenicity of influenza vaccination have concentrated on the humoral response. The humoral response is evaluated by determining the percentage of recipients who develop antibody titers that are considered protective against influenza (ie, ≥1:32), the percentage who develop a serological response (ie, >4-fold increase in titer), and the post-vaccination geometric antibody levels (**Keitel and Couch, 2002).**

Anar et al. (2010) Conducted a study investigated the antibody immune response against influenza vaccine and also the efficacy of vaccination on clinical findings in patients with Chronic Obstructive Pulmonary Disease (COPD) following influenza vaccination (vaccinated and unvaccinated COPD groups and healthy volunteers were also included in the study as a control group).

In that study Influenza A and B Ig M and Ig G antibody levels of COPD patients and healthy volunteers were measured before vaccination and were measured one month and one year after the vaccination. The results of that study concluded that seasonal influenza vaccination with the trivalent inactivated influenza vaccine especially in severe or very severe COPD patients who need hospitalization was evaluated as beneficial in clinical use. Also,no significant difference in the antibody immune response to seasonal trivalent inactivated influenza vaccine between vaccinated COPD patient and healthy volunteers was found.

It noteworthy that in the previous study blood samples were analyzed for influenza A and B IgM and IgG antibodies level by using microELISA kits (IBL, Immunobiological Laboratories, GmbH, Hamburg, Germany) which is the same kits used in our study.

Contrary to the results of the previous study, **Nath et al. (2014)** conducted a study on 20 COPD patients and 14 healthy controls received the trivalent inactivated influenza vaccine. Antibody titers (H1N1-vaccine specific antibodies) at baseline and 28 days post-vaccination were measured using the hemagglutination inhibition assay (HAI) assay. The aim of this study was to assess the immunogenicity of the trivalent influenza vaccine in persons with COPD compared to healthy controls without lung disease. In this observational study the result indicated that the humoral immune response to the trivalent inactivated influenza vaccine was lower in persons with COPD compared to non-COPD controls. Seroconversion, defined as a fold increase ≥ 4 in Ab titre between pre and four weeks post-vaccination, occurred in 90% of healthy controls, but only in 43% of COPD patients.

In our opinion there are limitations to the study by**Nath et al. (2014)**. Serological responses to only one of three viral strains present in the trivalent influenza vaccine were measured (The Influenza A/H1N1 subtype was the only strain used to assess Ab titres in that study). It is possible that antibody responses vary against the three viral strains of the vaccine. Additionally, the small sample size limits the statistical power of their observations.

Also in that study Serum H1N1-specific antibodies (Abs) were measured by haemagglutination inhibition (HAI) assay, while in our study influenza A and B antibodies were measured by ELISA.

It is noteworthy that the mean age of COPD patients recruited in that study is 66.2 years and that of normal subjects is 54.3 years, while in our study the mean age of the participating COPD group is 49.65 years. So these results that imply low post vaccination Ab titres in COPD individuals may not be directly related to COPD per se, rather than being related to age factor.

Most studies show that antibody responses to influenza vaccination decreases in older adults **(ACIP, 2013).**

Goodwin et al. (2006) performed a quantitative review of 31 vaccine antibody response studies conducted from 1986 to 2002 and compared antibody responses to influenza vaccine in groups of elderly versus younger adults. They showed that the antibody response in the elderly is considerably less than in younger adults (elderly adults were 2–4 times less likely to seroconvert or achieve protective HAI titers after vaccination, compared with younger adults).

These 31 studies were conducted from 1986 to 2002 in North America, Japan, Israel, and nine European countries. The “young” age groups included individuals aged 17–59. The “elderly” age groups included individuals aged 58–104 years, with a mean age ranging from 68 to 86 years.

Seidman et al. (2012) conducted a quantitative review of 60 articles published between 1987 and 2006 to identify determinants of serological responses to inactivated seasonal influenza vaccines including number of doses, adjuvant, and subject characteristics. The results showed that both children and elderly

tended to have lower immune responses compared to adults whereas use of adjuvant and a second vaccine dose tended to increase immune response. Pre-vaccination serological status had a large influence on the immune response to vaccination. Also they found substantial heterogeneity among studies, even with similar population settings and vaccination regimen.

Jahnz-Rózyk, et al. (2004) conducted a study on 42 asthmatics with a mean age of 46.6 years and 45 healthy controls with a mean age of 44.5 years who were vaccinated with a single dose of intramuscular inactivated seasonal influenza vaccine. The study aimed to compare the humoral antibody response to influenza vaccine in patients with allergic bronchial asthma and healthy controls. The humoral antibody response to haemagglutinin (HI) and neuraminidase (NI) glycoproteins of the three virus antigens in the vaccine were measured before and 6 weeks after vaccination.

The results indicated that there was a significant increase of the geometric mean titres **(**GMT) of HI and NI antibodies to the three virus antigens in both studied groups and there were no significant differences between the asthmatics and normal controls in response rate to vaccination.

The study concluded that the antibody immune response to influenza vaccine in asthmatics is comparable with those in normal individuals.

Romanowska et al. (2007) performed a study on 20 children with bronchial asthma vaccinated with split inactivated vaccine to assess humoral response to influenza. Response to influenza hemagglutinin was measured before vaccination and after 1 month by hemagglutination inhibition (HAI) test. Antibody titers were significantly higher after vaccination than before vaccination. The mean fold increase of antibody levels after vaccination was 12.2, 53.7 and 16.5 (for influenza antigen A/H1N1, A/H3N2 and B respectively). The post-vaccination percentage of patients with protective anti-hemagglutinin antibody titers ≥1:40 (protection rate) reached 95%, 100% and 100% which was 40%, 35% and 5% before vaccination (for influenza antigen A/H1N1, A/H3N2 and B respectively). The results showed the immunogenicity and safety of inactivated influenza vaccine in children with asthma.

A number of variables affect serum antibody responses to flu vaccine including vaccine composition, age, immunocompetence, prior antigenic priming and testing method used. Considerable variation in test results occurs between vaccines, years, different populations and laboratories; nevertheless, patterns of responses tend to be similar (**Couch, 2008).**

Based on the present data it can be concluded that despite the immune response to flu vaccine has been investigated in many studies, yet according to the best of our knowledge there is no available studies comparing the antibody immune response to flu vaccine between asthma and COPD patients, like our own work, therefore our results cannot be generalized due to lack of other comparable studies. So future studies in this area of search should be done to provide greater details on our search points and to allow us to evaluate our study results in comparison to others work. Also developing guidelines for basal and post vaccination induction of influenza antibody levels is desirable to evaluate the immunogenicity and efficacy of different types of flu vaccine in different population groups.

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