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CLINICAL STUDY

Immune response after a single vaccination against 2009 influenza A H1N1 in hemodialysis patients

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ABSTRACT

Background: Influenza infection is a significant cause of morbidity and mortality in general population. Hemodialysis patients are considered at high risk of influenza infection given their altered immune status. Pandemic influenza virus is new for human beings, so it is hard to predict the response to infection or vaccination. We aimed to evaluate the response to pandemic H1N1 vaccination in hemodialysis patients. *Methods*: A total of 70 patients on chronic hemodialysis and 20 controls who had been vaccinated against the pandemic influenza virus 5 weeks before the time of blood sampling were included into this study. The anti-H1N1 immunoglobulin G (IgG) antibodies of the patients were studied with enzyme immune assay (EIA) method. Our cut-off optical density (OD) value was 1.503. If the patient's OD value was equal or higher than this value, it was considered as positive. If it was lower, it was considered as negative. *Results*: The mean OD value was 2.22 ± 0.42 in the patient group and 1.99 ± 0.34 in the control group ($p < 0.05$). Two of 70 patients and 1 of the controls had negative OD values and they were considered as nonresponsive to vaccination. There was also a negative correlation between the age and OD values in the patient group ($r = -0.277$, $p < 0.05$). *Conclusion*: H1N1 vaccine can be performed safely and cost effectively with a single dose to the risk groups especially to the hemodialysis patients. Evaluation of H1N1 IgG antibody with enzyme-linked immunosorbent assay (ELISA) may be a safe, easy, and cost-effective assay.

Keywords: hemodialysis; immune response; pandemic influenza; vaccination

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INTRODUCTION

Influenza infection is a significant cause of morbidity and mortality in general population. The prevention of influenza infection as well as other infections in some special populations such as patients with chronic kidney disease (CKD) is one of the aims of public health practitioners, clinicians, and governments. Patients with CKD are considered at high risk of influenza infection given their altered immune status.[1]

After the report of first cases of influenza A (H1N1) virus infections in the spring of 2009 in United States and Mexico, the numbers of the H1N1 virus infections have increased like an avalanche. Subsequently, the World Health Organization (WHO) declared influenza pandemic in June 2009 and advised governments to prepare for a long-term battle against an unstoppable new flu virus. The rapid spread of a novel influenza A H1N1 virus with its potential to cause acute respiratory distress syndrome (ARDS) and death in healthy adults and pregnant and risk groups was probably the most important problem of clinicians, health authorities, and governments in 2009. This not only was a worldwide health problem but also carried great economical disruptions in health budgets of all countries. By 31 May, WHO had reported 15,510 cases in 53 countries, and over 99% of subtyped influenza A isolates in Europe and the United States have been pandemic H1N1 influenza A.[2,3]

Most people with H1N1 infections were children and young adults. Between the first report from the United States in 2009 and end of January 2010 more than 600 people died because of H1N1 infection in Turkey. Because of the possibility of the infection of healthy and young people and the potential of high contamination risk for the virus, the Turkish Health Ministry was alarmed and took some precautions against the infection. Undoubtedly, vaccination of the

risk groups and young citizens was the most important step of these precautions. CKD patients were also accepted as a risk group and almost all patients undergoing hemodialysis (HD) or peritoneal dialysis (PD) were vaccinated according to their wish and whether they had a greater risk for developing allergic sensitization to one of the ingredients of the vaccine.

There are very limited data on the risk factors to predict severe cases and deaths associated with this pandemic influenza. The presence of metabolic conditions (such as diabetes and obesity), respiratory disease, heart disease, other infections, kidney and liver disease has been shown to be associated with higher mortality.[4] Patients with CKD have a higher incidence of infectious diseases, which is thought to be related to an impaired T-cell activation by antigen-presenting cells.[5,6]

In patients on HD, vaccination response can be suboptimal.[7] Influenza vaccination may be less prone in stimulating serum antibodies of high avidity than natural infection.[8] Serum antibody response to the previous influenza vaccine virus was shown to decline with increasing age and uremia.[1,9] Pandemic influenza virus is new for human beings, so it is hard to predict the response to infection or vaccination and this subject is still controversial not only for health practitioners but also for politicians and budget planners of the governments. In this study, we aimed to evaluate the response to pandemic H1N1 vaccination in HD patients.

SUBJECT AND METHODS

Study design

The purpose of this study was to evaluate the immunogenicity of H1N1 vaccine in CKD patients and also in healthy adults between the ages of 16 and 90 years. We also tried to evaluate the relationship between the age and response to vaccination in chronic renal failure (CRF) patients. All authors contributed to the content of the manuscript, had full access to all study data, and vouch for the completeness and accuracy of the data. Because of the novelty of the pandemic H1N1 strain, we retrospectively collected data relating to the occurrence of select adverse events of special interest. These events included several neurologic, immune system, or any other disorders.

Patients and controls

A total of 70 patients (39 male and 31 female) on chronic HD and 20 healthy, nonpregnant controls (8 males and 12 females) who had been vaccinated against the pandemic influenza virus 5 weeks before the time of blood sampling were included in this study. Informed consent was taken from each patient and control, prior to the blood test. The study design was approved by the local ethics committee. Patients treated with immunosuppressive drugs or having a suspect of previous or newly onset H1N1 infection were not included in the study. Patients with a history of vasculitis, hematological or solid malignancy, and immune deficiency were excluded. The vaccine was injected into the musculus deltoideus of the contralateral arm of vascular dialysis access in the patient group. All of the patients and controls also had a history of seasonal influenza virus vaccination simultaneously with H1N1 vaccination. HD patients also had a routine annual vaccination history against the seasonal influenza with inactivated influenza vaccine (TIV), but controls did not have an annual vaccination against seasonal influenza previously. Any adverse events of special interest or serious adverse event was to be reported within 24 h or during the period since blood sampling.

H1N1 vaccine

In this vaccination program, pandemic influenza vaccine including at least 7.5 mg of hemagglutinin from the following recommended influenza virus strain, A/California/7/2009 (H1N1)v like strain (X-179A), was used (Focetria®, Novartis Vaccines and Diagnostics, Rosia-Sovicille (SI), Italy). The vaccine did not contain live virus particles. The active ingredients of the vaccine are purified viral proteins called hemagglutinin and neuraminidase, which were isolated from the surface of influenza virus particles and inactivated with formaldehyde. These viral proteins were prepared from the strain of influenza virus that complies with the WHO recommendations. The vaccine contains squalene as an adjuvant to stimulate a better response.

Laboratory assay

The anti-H1N1 immunoglobulin G (IgG) antibodies of the patients were studied with enzyme immune assay (EIA) method (Cusabio Biotech Co., Newark, NJ, USA). The sera of the patients were collected 5 weeks after the vaccination. We used a serum separator tube and allowed samples to clot for 30 min before centrifugation for 15 min at 1000*g*. We removed serum and stored them at –74°C until they were studied. We avoided repeated freeze–thaw cycles.

The kit was used according to the manufacturer's instructions. Briefly, 100 μL of positive control, negative control, and serum samples was added to the wells. The plate was covered with an adhesive strip and incubated for 30 min at 37°C. Then each well was aspirated and washed three times. After washing, 100 μL of horseradish peroxidase (HRP) anti-human IgG was added to each well, and the plate was incubated for

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30 min at 37°C. After this incubation, the plate was washed five times. Then 90 μL of tetramethylbenzidine (TMB) substrate was added to the wells, and the plate was incubated at 37°C for 20 min. Then 50 μL of stop solution was added and the optical density (OD) of each well was determined at 450 nm within 30 min. For the calculation of the anti-H1N1 IgG antibodies, OD of negative control is multiplied by 1.4 as described in the manual of the kit. According to this, our cut-off OD value was 1.503. If the patient's OD value was equal or higher than this value, it was considered as positive. If it was lower, it was considered as negative.

We used independent samples' test for comparing the mean values of two different groups and Pearson correlation test to evaluate the relationships between variables. We considered a p-value <0.05 as statistically significant.

RESULTS

Patient characteristics are shown in Table 1. The mean age of the patients was 57.88 ± 13.09 years and that of the controls 35.35 ± 10.04 years. The difference between the ages of the patients and controls was statistically significant ($p < 0.0001$). Sixteen of 70 patients were diabetic. There was no statistically significant difference about H1N1 IgG antibody levels between diabetics and nondiabetics. The mean OD value was 2.22 ± 0.42 in the patient group and 1.99 ± 0.34 in the control group. The difference between the two groups was statistically significant ($p < 0.05$). Two of 70 patients and 1 of the controls had negative OD values and they were considered as nonresponsive to vaccination. There was also a statistically significant negative correlation between the age and OD values in the patient group ($r = -0.277$, $p < 0.05$). Mean Kt/V result of the patients was 1.44 ± 0.15 and mean nPCR result was 0.97 ± 0.18 g/kg/day. Forty-two of the patients were having dialysis with high-flux (HF) membranes and 28 of them were having dialysis with low-flux (LF) synthetic membranes. Mean OD value was 2.27 ± 0.49 in HF users and 2.15 ± 0.29 IU/L in LF users. The difference between HF and LF users was not statistically significant. There were no significant correlations between H1N1 antibody levels and Kt/V or nPCR.

TABLE 1. Patients' characteristics.

Age (year)	57.88 ± 13.09
Male:female	39:31
Kt/V	1.44 ± 0.15
nPCR	0.97 ± 0.18
HF:LF	42:28
Diabetics:nondiabetics	16:54
OD value	2.22 ± 0.42

Note: nPCR, normalized protein catabolic rate; HF, number of high-flux membrane users; LF, number of low-flux membrane users; OD, optical density.

DISCUSSION

Antibody response which is induced by vaccination is a clinically relevant way to evaluate immune functions, as it reflects both antigen recognition and processing and humoral response. To the best of our knowledge, this study is the only study ever performed in HD patients evaluating antibody response with enzyme-linked immunosorbent assay (ELISA) after pandemic H1N1 vaccination. CKD is generally considered as an immunosuppressive state. Acquired immunity disturbances in CKD patients are caused by uremia, as well as the HD procedure, CRF complications, and the interventions for their treatment. The acquired immunity disturbances in CKD patients mainly involve T lymphocytes and antigen-presenting cells.[10] This is because there has been a predisposition to infections, and probably the most important, inadequate response to vaccinations using proteins like influenza virus hemagglutinin. The Advisory Committee on Immunization Practices (ACIP) recommended to receive annual influenza vaccination.[11] However, it is still controversial to perform a single-dose or booster vaccination. Song et al. reported that influenza vaccination of HD patients, according to current recommendations, resulted in an effective humoral immune response. Booster immunization did not further increase the proportion of HD patients with protective antibody titers.[12] In this last pandemic of twenty-first century, one of the polemics about H1N1 vaccination is how to perform vaccines: a single-dose or booster vaccination? In Turkey, it was recommended as just a single dose for young healthy adults, but two different doses for children under the age of 9 and for risk groups in which the second dose must be done at least 3 weeks after the first dose. HD patients were accepted as risk group. In our study, we found that a single-dose vaccination for H1N1 virus may be adequate for immunization in HD patients. Interestingly, IgG antibody levels for H1N1 virus in the patient group were higher than that in the healthy control group. It may seem as a suspicious result at the beginning, but it is important to remember that the HD patients were vaccinated annually against seasonal influenza after they

began HD but none of the controls have such a vaccination history. In a report, the Centers for Disease Control and Prevention (CDC) has suggested that vaccination with recent (2005–2009) seasonal influenza vaccines is unlikely to provide protection against the novel influenza A (H1N1) virus. Although vaccination of adults with seasonal TIV generally resulted in a small increase in antibodies against the novel influenza A (H1N1) virus, whether such levels of cross-reactive antibody provide any protection against infection with novel influenza A (H1N1) virus is unknown.[13] Alternatively, in another study, the importance of yearly influenza vaccination was emphasized, which may be advantageous not only for the corresponding season but also for the subsequent years.[14] In our study, we did not evaluate the antibody levels against seasonal influenza after annual vaccinations, but we found higher levels of IgG antibodies against H1N1 in the patient group than the healthy controls and this gave us courage about the immunization of HD patients both for annual influenza vaccination and for H1N1.

Another impressive result of our study was the negative correlation between IgG antibody levels and age in the patient group. We had to include young healthy adults into the control group because in the beginning of the vaccination program of Turkish Health Ministry for pandemic influenza, only the young adults and risk groups were accepted as candidates for vaccination. So, we could not find elderly healthy people who were vaccinated for the control group. Because of that, there was a statistically significant difference between the ages of the patient group and the controls. Our results revealed that the antibody response to pandemic influenza declined with age. Our results were similar to the experimental study of Pifer et al.[15] who showed the impaired antibody response in aged animals. This can be interpreted as follows: elderly people may need to have booster injection for adequate immunization.

Lonneman et al.[16] reported that HF HD membranes may improve T-cell functions compared to LF membranes. We did not find any difference about antibody response between HF membranes and LF membranes. Also, it was shown that patients receiving HD with ultrapure dialysates achieved higher antibody titers and seroconversion rates following hepatitis B virus (HBV) vaccination, compared to patients receiving HD with conventional dialysate.[17] In our study, all of the patients were dialyzed with ultrapure dialysate and perhaps this may have contributed to the excellent antibody response to vaccination in the patient group.

Our study was designed for ELISA. Determination of hemagglutination-inhibiting antibody titers may be the best way for evaluating the vaccine response, but it is sometimes hard to perform this method because of the lack of experienced medical practitioners. In this study, we performed the test approximately in 4 h and it seems to be reliable and cost effective. There may be some questions about the reliability and accuracy of this ELISA kit. For these questions, we made a self-control of ourselves. We evaluated the serum H1N1 IgG antibody levels in two patients who were previously known to have H1N1 virus infection, which was proven with polymerase chain reaction (PCR). We found high levels of IgG antibody against H1N1. We also evaluated a healthy control that did not pass H1N1 infection and was not vaccinated for pandemic influenza and found negative IgG levels against H1N1. We did not include the results of these people into the statistics and study. These were just for the verification of our study and the kit that we used.

In conclusion, as influenza, especially H1N1, may result in some medical complications leading to death and also because of high treatment costs; HD patient groups should be vaccinated yearly for seasonal influenza and in addition they must be vaccinated against H1N1 virus. H1N1 vaccine can be administered safely and cost effectively with a single dose to the risk groups, especially to the HD patients. Trial of different vaccination protocols such as booster vaccination may not be necessary for increasing antibody response in HD patients except elderly patients. Evaluation of H1N1 IgG antibody with ELISA can be a safe, easy, and cost-effective assay.

Declaration of interest: None of the authors have any conflict of interests to declare.

Corrigendum: At the point of original online publication, Dr Sultan Ozkurt's surname was misspelled Ozturk. This has been corrected in the print edition.

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