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Immunogenicity of influenza A(H1N1)pdm09 vaccine in patients with diabetes mellitus

With special reference to age, body mass index, and HbA1c

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Abbreviations: DM, diabetes mellitus; HbA1c, hemoglobin A1c; ACIP, Advisory Committee on Immunization; WHO, World Health Organization; HAI, hemagglutination-inhibition; GMT, geometric mean titer; GMTR, geometric mean titer ratio; OR, odds ratio; CI, confidence interval

Subjects with diabetes mellitus are considered to be at high risk of influenza infection and influenza-associated complications. To evaluate the immunogenicity of the influenza A(H1N1)pdm09 vaccine among these subjects, we performed a prospective cohort study and measured hemagglutination inhibition antibody titers at baseline and 3 weeks after vaccination in 49 patients. No serious adverse events were reported. We were able to perform analyses for 48 patients, after excluding one patient with suspected infection. The vaccine induced a rise of about 9-fold in the mean antibody level. The sero-response proportion was 79%, and the sero-protection proportion was 73%. Patients with older age and lower body mass index tended to show lower immune response. Multivariate analysis indicated an independent negative effect of hemoglobin A1c level on the sero-protection proportion. A single A(H1N1)pdm09 vaccination achieved a sufficient level of immunity among diabetic patients, but both clinicians and patients should be aware of the potential for reductions in immune response.

Introduction

Patients with diabetes mellitus (DM) are presumed to have abnormalities in immune function and are classified as a high-risk group for developing complications, hospitalizations and death related to influenza.[1-3] According to the Advisory Committee on Immunization Practices (ACIP) in the United States, vaccinating high-risk individuals before influenza season each year is the most effective measure for reducing the impact of influenza.[4] Annual influenza vaccination has therefore long been recommended for these individuals.[1,5]

In June 2009, the World Health Organization (WHO) declared a global pandemic of the influenza A(H1N1)pdm09 and identified chronic medical conditions as being specific risks for infection.[6] As a result, many diabetic patients received H1N1 vaccination according to the recommendations of the WHO.[7] However, these recommendations were based on clinical trials in healthy individuals,[8] and little is known about the immunogenicity of the vaccine in high-risk groups, including diabetic patients. The present study investigated immunogenicity of the vaccine in diabetic individuals and tried to identify factors affecting immune response.

Results

Study subjects

We excluded 1 patient in whom both pre- and post-vaccination titers were 1:160, as subclinical infection was suspected in that patient. Among the total of 48 diabetic patients, 7 patients with type 1 DM (3 men, 4 women; mean age (± standard deviation), 47.3 ± 14.6 y) and 41 patients with type 2 DM (33 men, 8 women; mean age, 59.8 ± 11.4 y) were analyzed. Subject characteristics are shown in **Table 1**. Mean hemoglobin (Hb)A1c level was 7.44%, and more than half of the patients were treated with insulin. No patients were receiving steroid therapy or undergoing dialysis. Type 1 patients were younger and the proportion of males was lower compared with type 2 patients. The distribution of HbA1c levels and body mass index (BMI) did not differ between groups.

Immune response

Results of antibody response by background factors are summarized in **Table 2**. The vaccine induced a mean increase in hemagglutination-inhibition (HAI) antibody level of 9-fold ($P < 0.0001$). Sero-response proportion was 79% (95% CI, 62–97%), and the sero-protection proportion was 73% (95% CI, 60–86%). The corresponding sero-conversion proportion

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Table 1. Characteristics of study participants

Characteristics	Study subjects				
	n (%)	Total (N=48)	Type 1 (N=7)	Type 2 (N=41)	*P* value
Sex	male	36 (75)	3 (43)	33 (81)	0.043
Age (y)	mean (S.D.)	57.9 (12.5)	47.3 (14.6)	59.8 (11.4)	0.029
	median (range)	61.0 (26–75)	45.0 (26–66)	62.0 (28–75)	
Duration of disease(y)	mean (S.D.)	7.0 (15.0)	8.0 (8.2)	12.9 (10.0)	0.235
	median (range)	9.5 (1–37)	5.0 (3–26)	10.0 (1–37)	
HbA1c	mean (S.D.)	7.44 (1.45)	7.81 (1.06)	7.37 (1.51)	0.160
	median (range)	7.30 (4.9–14.4)	8.20 (5.9–9.0)	7.30 (4.9–14.4)	
Body mass index (kg/m^2)	mean (S.D.)	23.8 (3.3)	23.0 (2.1)	23.9 (3.5)	0.365
	median (range)	23.5 (17.0–32.2)	22.2 (20.8–27.3)	23.5 (17.0–32.2)	
Treatment of DM	no medication	5 (10)	0 (0)	5 (12)	
	internal use only	16 (33)	0 (0)	16 (39)	
	insulin +	27 (56)	7 (100)	20 (49)	
Treatment of hypertension	received	21 (44)	1 (14)	20 (49)	0.096
Treatment of hyperlipidemia	received	17 (35)	2 (29)	15 (37)	0.698
Steroid therapy	received	0	0	0	
Dialysis	received	0	0	0	

was 73% (95% confidence interval [95% CI], 60–86%). Older patients showed a smaller immune response, as reflected in post-vaccination geometric mean titer (GMT) (P = 0.027) and sero-protection proportion (P = 0.059). Lower BMI was associated with lower sero-response proportion, displaying a clear dose-response relationship (P = 0.006). This relationship remained unchanged (trend P = 0.008) even after considering the effects of potential confounders (**Table 3**). The odds ratio (OR) for sero-response among those subjects with highest HbA1c (≥7.6%) was low, although no significant relationship was apparent.

Predictors of immune response in terms of sero-protection proportion were also analyzed (**Table 4**). Older age was suggested to be related to lower sero-protection with marginal significance in the crude model. This relationship appeared significant in Model 2, which involved age, HbA1c level and BMI (trend P = 0.033). In addition, subjects with the highest HbA1c level (≥7.6%) tended to show a lower sero-protection proportion (crude OR, 0.39; 95% CI, 0.06–2.42) than subjects with the lowest HbA1c level (<6.5%), although this difference was not significant. After adjusting for potential confounders, we found that a higher HbA1c was independently associated with lower sero-protection with marginal significance (Model 1: trend P = 0.071; Model 2: trend P = 0.074). In addition, subjects with lower BMI showed a decreased OR for sero-protection (trend P = 0.079).

These findings suggested that (1) older age may be related to poorer antibody response as reflected in post-vaccination GMT and sero-protection rate, (2) lower BMI seemed to be associated with lower sero-response and sero-protection, and (3) higher HbA1c level might have affected immune response, showing lower ORs for sero-response and sero-protection.

To explore these findings in more detail, we conducted stratified analyses. In the analyses in which effects of age and HbA1c (**Table 5**, A), and effects of BMI and HbA1c level (**Table 5**, B) were examined, higher HbA1c still induced lower immune response, although significant relationships could be detected in only part of the trends. Particularly among older patients (≥61 y), higher HbA1c was significantly associated with lower GMT ratio (GMTR), fold rise, and sero-protection proportion (P = 0.043, P = 0.044, and P = 0.043 for each). Similar relationships were also suggested among patients with higher BMI (≥23.5 kg/m^2).

Discussion

The influenza A(H1N1)pdm09 virus was reported to be distinct from seasonal human A(H1N1).[8] The pre-vaccination antibody titer of every subject we analyzed was <1:40 in the present study. This situation facilitated the evaluation of immunogenicity. We showed that a single 15-μg dose of unadjuvanted A(H1N1) pdm09 vaccine induced sufficient antibody among patients with DM. This immunity was sufficient to meet the international criteria of the European Agency for the Evaluation of Medical Products and the US Food and Drug Administration. However, the sero-protection proportion among subjects (73%) was slightly lower than reported proportions in healthy adults (79–95%).[7,9,10] In particular, the proportion among patients >65-y-old (58%) was rather lower than the reported proportions in age-matched healthy adults (79–80%).[9,10] No serious adverse effects were observed and all reported adverse reactions were self-limited.

This study also investigated factors that may affect immunogenicity of the vaccination. We found that the following

factors might have induced lowered immunogenicity—older age, lower BMI, and higher HbA1c level. Decreased immune response in the elderly has been reported in previous studies of A(H1N1) pdm09 vaccine[10-12] and seasonal influenza vaccine.[13-15] Compromised nutritional status[16-18] and decreased T-cell activity[18-20] could be contributing factors for that finding. One study in patients with hepatitis C reported a decreasing effect of lower BMI on immune response to A(H1N1)pdm09 vaccine.[11] Another study in the elderly reported that a combination of BMI ≤ 18.5 kg/m^2 and loss of more than 5% of body weight in 6 mo found to be significantly associated with poor immune response.[21] Although the mechanisms remain unclear, nutritional status and physical strength might be involved in decreased immune response.

To the best of our knowledge, no studies have reported the effects of HbA1c on immune response. Originally, few clinical trials examined the efficacy of influenza vaccination in patients with DM. One previous study suggested an impaired immune response in patients with poorly controlled diabetes.[22] Another study in patients with well-controlled diabetes showed that humoral and cell-mediated immune responses to seasonal influenza vaccine were normal and immune response did not differ from those observed in age-matched normal subjects.[23] A third study showed that diabetic patients in the older age range or with longer disease duration showed a lower sero-conversion proportion with A(H1N1)pdm09 vaccine.[24] Such results are broadly consistent with our findings that immunity was sufficient as a whole, but older age, decreased BMI and increased HbA1c level were associated with poor immunogenicity. The reason for these minor differences is unclear, but differences in the severities of DM and the comorbidities or genetic characteristics of the population may have been involved. In stratified analysis, older patients with poorer HbA1c showed rather lower immune response. Although the sample size of the present study was small, the results might be useful in addressing this point. Larger studies are needed to confirm the present findings.

Table 2. Immuno responses to monovalent 2009 influenza A(H1N1) vaccine among diabetic patients (continu

		Geometric mean*		Fold rise*	Postvac titer**	
					≥4-fold rise	≥1:40
Category	N	Pre vac	Post vac		n (%)	n (%)
Entire sample	48	6	53	9 ($P < 0.0001$)	38 (79)	35 (73)
Sex						
Male	36	6	44	8 ($P < 0.0001$)	28 (78)	25 (69)
Female	12	7	95	13($P < 0.0001$)	10 (83)	10 (83)
		($P = 00.101$)	($P = 00.124$)	$P = 0.254$)	($P = 00.682$)	($P = 00.348$)
Age						
<57	15	8	96	12($P < 0.0001$)	12 (80)	13 (87)
57–64	14	6	62	11($P < 0.0001$)	12 (86)	11 (79)
65+	19	5	30	6($P < 0.0001$)	14 (74)	11 (58)
		($P = 0.021$)	($P = 0.027$)	($P = 0.3201$)	($P = 0.624$)	($P = 0.059$)
DM subtype						
Type 1	7	6	54	9($P = 0.0034$)	5 (71)	5 (71)
Type 2	41	6	53	9($P < 0.0001$)	33 (80)	30 (73)
		($P = 0.812$)	($P = 0.870$)	($P = 1.000$)	($P = 0.585$)	($P = 0.924$)
Duration of disease (y)						
<10	24	6	55	9 (P < 0.0001)	19 (79)	17 (71)
10+	24	6	52	9 (P < 0.0001)	19 (79)	18 (75)
		($P = 0.132$)	($P = 0.900$)	($P = 0.505$)	($P = 1.000$)	($P = 0.745$)
HbA1c (%)						
<6.5	10	6	65	11 ($P = 0.0007$)	8 (80)	8 (80)
6.5–7.5	20	6	61	11(P < 0.0001)	17 (85)	16 (80)
7.6+	18	7	42	6 (P < 0.0001)	13 (72)	11 (61)
		($P = 0.115$)	($P = 0.243$)	($P = 0.198$)	($P = 0.529$)	($P = 0.222$)
Body mass index (kg/m2)						
<22.1	13	6	34	6 ($P = 0.0006$)	8 (62)	9 (69)
22.1–23.8	16	6	50	9 (P < 0.0001)	11 (69)	10 (63)
23.9+	19	6	77	12 (P < 0.0001)	19 (100)	16 (84)
		($P = 0.806$)	($P = 0.454$)	($P = 0.221$)	($P = 0.006$)	($P = 0.296$)
Treatment of DM						
No medication	5	6	70	12 ($P = 0.0491$)	4 (80)	4 (80)
Internal use only	16	7	50	7 (P < 0.0001)	11 (69)	10 (63)
Insulin +	27	6	53	9 (P < 0.0001)	23 (85)	21 (78)
		($P = 0.472$)	($P = 0.846$)	($P = 0.674$)	($P = 0.410$)	($P = 0.649$)
Insulin-	21	7	54	8 (P < 0.0001)	15 (71)	14 (67)
Insulin+	27	6	53	9 (P < 0.0001)	23 (85)	21 (78)
		($P = 0.319$)	($P = 0.841$)	($P = 0.624$)	($P = 0.244$)	($P = 0.390$)

*Wilcoxon signed–rank test for intra-category comparisons, and the Wilcoxon rank sum test for inter-category comparisons. **χ^2 test between 2 categories and the Mantel-extension method for trend test among 3 categories.

Table 2. Immuno responses to monovalent 2009 influenza A(H1N1) vaccine among diabetic patients (continued)

		Geometric mean*		Fold rise*	Postvac titer**	
					≥4-fold rise	≥1:40
Category	N	Pre vac	Post vac		n (%)	n (%)
Treatment of hypertension						
None	27	6	54	9 (P < 0.0001)	21 (78)	20 (74)
Received	21	6	52	9 (P < 0.0001)	17 (81)	15 (71)
		(*P* = 0.580)	(*P* = 0.882)	(*P* = 0.875)	(*P* = 0.788)	(*P* = 0.838)
Treatment of hyperlipidemia						
None	31	6	57	9 (P < 0.0001)	24 (77)	24 (77)
Received	17	6	47	8 (P < 0.0001)	14 (82)	11 (65)
		(*P* = 0.457)	(*P* = 0.428)	(*P* = 0.914)	(*P* = 0.687)	(*P* = 0.343)
Prevaccination titer						
<1:10	37	5	45	9 (P < 0.0001)	30 (81)	26 (70)
1:10–1:20	11	12	97	8 (*P* = 0.0004)	8 (73)	9 (82)
		(*P* <0.0001)	(*P* = 0.169)	(*P* = 0.321)	(*P* = 0.549)	(*P* = 0.449)

*Wilcoxon signed–rank test for intra-category comparisons, and the Wilcoxon rank sum test for inter-category comparisons. **χ^2 test between 2 categories and the Mantel-extension method for trend test among 3 categories.

Better methods to improve immunogenicity among subjects with poor immune response clearly need to be considered. A second vaccination might be effective. Previous studies[25-29] have suggested that 2 doses of vaccine are required to elicit a protective immune response in populations that are immunologically naïve to a new influenza strain. One study[10] reported that immune responses in the elderly could be substantially boosted by a second dose of vaccine—among subjects ≥61-y-old who received a 15-μg dose of unadjuvanted A(H1N1)pdm09 vaccine, the sero-protection proportion was 79.1% at 21 d after the first dose, and 93.3% at 14 d after the second dose (35 d after first dose). According to our data, the proportion at 21 d after vaccination among diabetic patients >65-y-old was substantially lower (58%) and a second dose might improve immunogenicity. In addition, adjuvants might be of help. Adjuvants are used to augment cellular and humoral responses by attracting greater numbers of antigen-presenting cells to the vaccination site. According to another study[30] that performed a randomized trial in healthy adults and older individuals to evaluate the immunogenicity and safety of A(H1N1)pdm09 vaccine at varying dosages of hemagglutinin with and without adjuvants, GMTs were higher in the adjuvanted groups than with the 15-μg unadjuvanted group in both age groups. And only 61% of participants achieved sero-protection after a single dose of the 15-μg unadjuvanted vaccination, whereas 81% achieved this state after a single dose of the 7.5-μg adjuvanted vaccination. The proportion increased to 94% after a second dose in the adjuvanted group, but remained low (68%) in the unadjuvanted group. Another study that performed a similar randomized trial of A(H1N1)pdm09 vaccine also reported that the addition of MF59 adjuvant to the vaccine increased the speed and magnitude of antibody response.[31] A second vaccination with adjuvants might thus represent a better method for diabetic subjects with poor immune response. Further studies are needed to verify this possibility.

Several limitations must be considered when interpreting the results of this study. First, the investigation was conducted in a single university hospital. Our study population comprised relatively well-controlled, adequately nourished patients without serious comorbidities, which may well have influenced the generalizability of the results. In addition, the number of subjects was small, limiting the study power. Second, we evaluated immunogenicity by antibody response only. Cell-mediated immunity to A(H1N1)pdm09 vaccine also needs to be investigated to elucidate the mechanisms of diminished response among patients with older age, lower BMI or higher HbA1c level. Finally, serum samples were collected only twice (before and 3 wk after vaccination) and we did not measure serum antibody responses after the pandemic season. We therefore had no data regarding the subsequent maintenance of immunogenicity, which represents a shortcoming of this study. In addition, we were unable to monitor subjects for clinical occurrences of influenza infection or influenza-like illness during the year. We were therefore unable to evaluate the actual effects of A(H1N1)pdm09 vaccine in protecting subjects. This represents another limitation of the current study. However, this is the first report on the effects of HbA1c on immune response to influenza A(H1N1)pdm09 vaccine. The independent negative effect of HbA1c we showed in this study is noteworthy to promote awareness regarding the potential for low immunogenicity in patients with poorly controlled diabetes.

In conclusion, we found that a single dose of A(H1N1)pdm09 vaccine safely induced a sufficient level of immunity and met international criteria in patients with DM. No severe adverse events were encountered. However, older age, lower BMI, and higher HbA1c were associated with reduced immune responses. Our results showed that older patients with higher HbA1c levels should be followed particularly carefully. Further studies are needed to clarify the mechanisms involved. To minimize influenza-related morbidity and mortality in the case of future influenza pandemics, it is important to determine the most effective methods for developing protective titers among patients with DM.

Patients and Methods

Study subjects

Study subjects were 49 patients with DM who visited the department of diabetes at Osaka City University Hospital for clinical follow-up. All subjects provided written informed

Table 3. Association between selected characteristics and sero-response proportion (≥4-fold rise)

			Crude analysis			Multivariate model*		
Category	N	n (%)	OR	(95%CI)	*P* value	OR	(95% CI)	*P* value
Sex								
Male	36	28 (78)	1.00			1.00		
Female	12	10 (83)	1.43	(0.26–7.89)	0.683	9.28	(0.22–390)	0.243
Age								
<57	15	12 (80)	1.00			1.00		
57–64	14	12 (86)	0.50	(0.21–10.6)	0.685	0.28	(0.01–6.12)	0.417
65+	19	14 (74)	0.70	(0.14–3.56)	0.667	0.63	(0.04–9.79)	0.738
			(Trend *P* = 0.622)			(Trend *P* =0.666)		
Type								
Type 1	7	5 (71)	1.00			1.00		
Type 2	41	33 (80)	1.65	(0.27–10.1)	0.588	2.59	(0.05–149)	0.645
Duration of disease (years)								
<10	24	19 (79)	1.00			1.00		
10+	24	19 (79)	1.00	(0.25–4.03)	1.000	0.48	(0.04–5.92)	0.567
HbA1c (%)								
<6.5	10	8 (80)	1.00			1.00		
6.5–7.5	20	17 (85)	1.42	(0.20–10.2)	0.730	0.97	(0.06–15.3)	0.985
7.6+	18	13 (72)	0.65	(0.10–4.18)	0.650	0.13	(0.005–3.51)	0.223
			(Trend *P* = 0.527)			(Trend *P* = 0.243)		
Body mass index (kg/m2)								
<22.1	13	8 (62)	1.00			1.00		
22.1–23.8	16	11 (69)	1.38	(0.30–6.40)	0.685	4.30	80.35–52.6)	0.254
23.9+	19	19 (100)	N.A.			N.A.		
			(Trend *P* =0.012)			(Trend *P* =0.008)		
Treatment of DM								
Insulin-	21	15 (71)	1.00			1.00		
Insulin+	27	23 (85)	1.52	(0.57–4.06)	0.409	4.22	(0.26–70.3)	0.316
Prevaccination titer								
<1:10	37	30 (81)	1.00			1.00		
1: 10–1:20	11	8 (73)	0.62	(0.13–2.96)	0.551	0.26	(0.01–6.02)	0.403

Logistic regression model. *Model included all variables in the table.

consent after the nature and possible consequences of the study had been explained. The study protocol was approved by the ethics committee at Osaka City University Graduate School of Medicine and was performed in accordance with the Declaration of Helsinki. None of the applicants met the exclusion criteria for eligibility, including history of 2009 influenza A (H1N1) infection, acute febrile illness or signs of severe acute illness at the time of vaccination, history of anaphylaxis due to vaccine components, or other condition contraindicating vaccination. In November 2009, subjects were administered a single dose of monovalent inactivated unadjuvanted split-virus 2009 pH1N1 vaccine containing 15 μg/0.5 mL of hemagglutinin antigen (Lot. HP01A; BIKEN). The vaccine contained thimerosal. The seed virus was prepared from reassortant vaccine virus A/California/7/2009 NYMC X-179A (New York Medical College), distributed by the Centers for Disease Control and Prevention in the United States. The vaccine was prepared in embryonated chicken eggs using standard methods for the production of seasonal trivalent inactivated vaccine.

Information collection

Before vaccination, subjects completed a self-administered questionnaire asking about sex, age at vaccination, date of birth, and comorbid diseases. In addition, one of the investigators extracted the following patient background and clinical information from the medical records—height, weight, DM subtype, treatment for DM; hypertension, hyperlipidemia,

Table 4. Association between selected characteristics and sero-protection proportion (titer ≥ 1:40)

			Crude analysis			Multivariate model1*			Multivariate model2**		
Category	N	n(%)	OR	(95%CI)	*P* value	OR	(95%CI)	*P* value	OR	(95%CI)	*P* value
Sex											
Male	36	25 (69)	1.00			1.00					
Female	12	10 (83)	2.20	(0.41–11.8)	0.356	1.74	(0.12–24.4)	0.681			
Age											
<57	15	13 (87)	1.00			1.00			1.00		
57-64	14	11 (79)	0.56	(0.08–4.01)	0.567	0.28	(0.02–3.86)	0.343	0.22	(0.02–2.24)	0.202
65+	19	11 (58)	0.21	(0.04–1.21)	0.081	0.16	(0.02–1.57)	0.116	0.09	(0.01–0.77)	0.028
			(Trend *P* = 0.066)			(Trend *P* = 0.137)			(Trend *P*=0.033)		
Type 1	7	5 (71)	1.00			1.00					
Type 2	41	30 (73)	1.09	(0.18–6.47)	0.924	1.10	(0.04–28.0)	0.956			
Duration of disease (years)											
<10	24	17 (71)	1.00			1.00					
10+	24	18 (75)	1.24	(0.35–4.43)	0.746	1.13	(0.21–6.12)	0.886			
HbA1c (%)											
<6.5	10	8 (80)	1.00			1.00			1.00		
6.5–7.5	20	16 (80)	1.00	(0.15–6.67)	1.000	0.93	(0.09-–9.32)	0.948	1.22	(0.15–10.1)	0.853
7.6+	18	11 (61)	0.39	(0.06–2.42)	0.313	0.10	(0.007–1.45)	0.091	0.16	(0.02–1.57)	0.115
			(Trend *P* =0.224)			(Trend *P* =0.071)			(Trend *P* =0.074)		
Body mass index (kg/m²)											
<22.1	13	9 (69)	1.00			1.00			1.00		
22.1-23.8	16	10 (63)	0.74	(0.16–3.50)	0.705	1.27	(0.17–9.24)	0.815	1.02	(0.17–6.16)	0.980
23.9+	19	16 (84)	2.37	(0.43–13.0)	0.321	9.17	(0.85–99.2)	0.068	7.15	(0.84–60.6)	0.071
			(Trend *P* =0.295)			(Trend *P* =0.078)			(Trend *P* =0.079)		
Treatment of DM											
Insulin-	21	14 (67)	1.00			1.00					
Insulin+	27	21 (78)	1.75	(0.49–6.31)	0.393	1.69	(0.25–11.6)	0.592			
Prevaccination titer											
<1:10	37	26 (70)	1.00			1.00					
1: 10-1:20	11	9 (82)	1.90	(0.35–10.3)	0.454	2.92	(0.25–34.8)	0.397			

Logistic regression model. *Model included all variables in the table. **Model included age, HbA1c and BMI.

steroid use, and laboratory data. We determined HbA1c levels using a Japan Diabetes Society (JDS)-certified method.

Serum collection and antibody titer measurement

Serum samples were collected twice: before vaccination and 3 wk after vaccination. Serum antibody titer against the vaccine strain was measured using the HAI assay according to standard methods using chicken erythrocytes.[10,32] All samples were assayed at the same time at the Surveillance Center Research Institute for Microbial Disease at Osaka University at April 2010.

Statistical analysis

We analyzed 48 patients, excluding 1 patient with suspected infection

To compare baseline characteristics between patients with type 1 and type 2 DM, we used the chi-square test for categorical variables and Wilcoxon rank-sum test for continuous variables. For assessment of the immunogenicity of influenza vaccine, the following outcomes were calculated—GMT, GMTR, fold rise, sero-response proportion (≥4-fold rise), and sero-protection proportion (post-vaccination titer ≥ 1:40). A titer <1:10 was regarded as 1:5 for the purpose of calculations. Reciprocal antibody titers were analyzed after logarithmic transformation. All results are presented in the original scale by calculating the antilogarithm.

Data were stratified for analysis by sex, age (<57 y, 57–64 y, or ≥65 y), DM subtype, duration of DM (<10 y or ≥10 y), HbA1c level (<6.5%, 6.5–7.5%, or ≥7.6%), body mass index (BMI) (<22.1 kg/m^2, 22.1–23.8 kg/m^2, or ≥23.9 kg/m^2), treatment of DM (no medication, internal use only or insulin

Table 5.

A. Stratified immunogenicity analysis by age and HbA1c					
				seroresponse**	seroprotection**
HbA1c	N	GMTR*	Fold rise*	n (%)	n (%)
Age < 61					
<6.5	4	12.3	7	3(75)	3(75)
6.5–7.5	9	33.9	17	8(89)	8(89)
7.6+	9	17.8	9	7(78)	8(89)
		P = 0.390	*P* = 0.421	*P* = 0.947	*P* = 0.573
Age ≥ 61					
<6.5	6	30.8	16	5(83)	5(83)
6.5-7.5	11	11.5	8	9(82)	8(73)
7.6+	9	4.2	4	6(67)	3(33)
		P = 0.043	*P* = 0.044	*P* = 0.427	*P* = 0.043
B. Stratified immunogenicity analysis by BMI and HbA1c					
				seroresponse**	seroprotection**
HbA1c	N	GMTR*	Fold rise*	n (%)	n (%)
BMI<23.5					
<6.5	6	11.0	6	4 (67)	4 (67)
6.5–7.5	11	23.8	10	9 (82)	8 (73)
7.6+	6	7.3	4	2 (33)	3 (50)
		P = 0.379	*P* = 0.364	*P* = 0.236	*P* = 0.553
BMI ≥ 23.5					
<6.5	4	42.0	32	4 (100)	4 (100)
6.5–7.5	9	18.9	12	8 (89)	8 (89)
7.6+	12	12.8	8	11 (92)	8 (67)
		P = 0.061	*P* = 0.084	*P* = 0.723	*P* = 0.109

*Kruskal–Wallis test. **Mantel–extension method.

use), treatment of hypertension (no medication or internal use), treatment of hyperlipidemia (no medication or internal use), and prevaccination titer (<1:10 or 1:10–1:20). The significances of GMT, GMTR, and fold rise within a category were assessed using the Wilcoxon signed–rank test, and intercategory comparisons were made using the Wilcoxon rank–sum test or Kruskal–Wallis test. The chi-square test and Mantel-extension trend test were also used where appropriate.

In addition, we calculated OR and 95% CI using logistic regression modeling to evaluate the independent effects of potential confounders. The models were constructed using sero-response or sero-protection as the dependent variable, and potential predictors such as sex, age, DM subtype, duration of DM, HbA1c level, BMI, treatment of DM and prevaccination titer as explanatory variables. From these models, we also constructed a reduced model using potential predictors (age, HbA1c level, and BMI) which showed *P* values or trend *P* values < 0.2 as explanatory variables. The sero-response proportion among patients with highest BMI (≥23.9) was 100%, which was why we used the group with lowest BMI (<22.1) as the reference stratum.

Two-sided *P* values less than 0.05 were considered significant. All analyses were performed using SAS version 9.3 software (SAS Institute).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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