at 32 weeks of gestation. In this case, the neonate' s HPA-5b alloimmunization had substantially less severe manifestations than other HPA-antigen alloimmunizations; the reported incidence of intracranial hemorrhage due to HPA-5b alloimmunization is 0-8% [7]. However, due to the risk of occurrence of NAIT, cordocentesis was performed at 34 weeks of gestation in order to decide the mode of delivery. The platelet count was sufficient for vaginal delivery. The fetus was delivered by vaginal birth, and there were no bleeding complications. The fetal platelet count transiently decreased, but subsequently increased without any treatment.

There must be a 50% risk of feto-maternal incompatibility of HPA-5b about next pregnancy because the patient has anti-HPA-5b antibody and her husband has a HPA-5 (a+b+) antigen. Recently, maternally administrated intravenous immunoglobulin has been the most successful therapy [6] however health insurance adaptation cannot be accepted in our country. In any case, a sufficient counseling about a recurrence of NAIT is important.

Acknowledgement

We would like to thank Dr. Hironori Kobayashi, Dr. Shoji Morita and Dr. Susumu Inoue (Japanese Red Cross Saitama Blood Center) for HPA typing.

References

1. Enomoto T, Maruoka H, Hanagaki S, Morita S, Shimamura M, Hando K, Ishijima A, Yoshioka A, Yuasa S, Takahashi K, Ohto H. 2000. Pregnancy-induced alloimmunization against platelet antigens: HLA and human platelet antigens (HPA). Jpn J Transfus Med. 46: 467-473.
2. Nagao N, Taniue A, Tomita N, Tomita T, Okubo Y, Ban C, Shibata Y. 1998. Neonatal alloimmune thrombocytopenia due to anti-HPA and the incidence of HPA antibodies in pregnant Japanese women. Jpn J Transfus Med. 44: 317-321.
3. Ohto H, Miura S, Ariga H, Ishii T, Fujimori K, Morita S. 2004. The natural history of maternal immunization against foetal platelet alloantigens. Transfus Med. 14: 399-408.
4. Silver RM, Porter TF, Branch DW, Esplin MS, Scott JR. 2000. Neonatal alloimmune thrombocytopenia: antenatal management. Am J Obstet Gynecol. 182: 1233-1238.
5. Overton TG, Duncan KR, Jolly M, Letsky E, Fisk NM. 2002. Serial aggressive platelet transfusion for fetal alloimmune thrombocytopenia: platelet dynamics and perinatal outcome. Am J Obstet Gynecol. 186: 826-831.
6. Berkowitz RL, Lesser ML, McFarland JG, Wissert M, Primiani A, Hung Crystal, Bussel JB. 2007. Antepartum treatment without early cordocentesis for standard-risk alloimmune thrombocytopenia. Obstet Gynecol. 110: 249-255.
7. Paladini D, Maruotti GM, Sglavo G, Fratellanza G, Quarantelli M, Martinelli P. 2007. Massive fetal hemorrhage and fetomaternal alloimmune thrombocytopenia from human platelet antigen 5b incompatibility: an unusual association. Ultrasound Obstet Gynecol. 29: 471-474.

AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 25, Number 4, 2009

DOI: 10.1089/aid.2008.0202

Progressive Renal Tubular Dysfunction Associated with Long-Term Use of Tenofovir DF

Ei Kinai and Hideji Hanabusa

Abstract

It became evident that tenofovir DF (TDF) causes a modest and gradual decline in GFR, however, the impact of long-term use of TDF on tubular function has not been fully evaluated. In 40 patients treated with TDF and 23 patients treated with other NRTIs, urine β_2-microglobulin (U-BMG), percentage tubular reabsorption of phosphate (%TRP), alkaline phosphatase (ALP), serum creatinine, and calculated GFR were prospectively measured for 96 weeks. In patients receiving TDF, median U-BMG rose from 188 μg/liter at baseline to 555 μg/liter at week 96 ($p = 0.02$), median %TRP declined from 94% at baseline to 90% at week 96 ($p = 0.002$), median ALP ratio compared with baseline persistently increased from 1 to 1.278 at week 96 ($p = 0.001$), and serum creatinine showed significant but minimal change from 0.64 mg/dl to 0.74 mg/dl at week 96 ($p = 0.02$). The GFR level declined minimally but significantly in TDF-receiving patients (–17 ml/min/1.73 m^2), whereas it did not change in other NRTI-receiving patients [+3 ml/min/1.73 m^2; mixed models analysis of variance (MMANOVA) $p = 0.03$ for overall change from baseline to week 96]. U-BMG, %TRP, ALP, or serum creatinine did not change significantly in other NRTI-receiving patients during the observation period. In five patients with marked changes in U-BMG (>10,000 μg/liter) and %TRP (<80%), both U-BMG and %TRP immediately recovered in all patients after discontinuing TDF, whereas GFR levels did not fully recover for 6 months in three patients. Prolonged treatment with TDF caused progressive renal tubular dysfunction as well as a modest decline in GFR. If U-BMG levels >10,000 μg/liter and %TRP values <80% are observed, discontinuing TDF may be beneficial.

Introduction

TENOFOVIR DISOPROXIL FUMARATE (TDF), a nucleotide analogue of adenosine 5′-monophosphate, is one of the most widely used antiretroviral agents for HIV-1-infected patients. Although clinical trials have concluded that TDF-associated renal toxicity is rare and reversible,[1–3] it is evident that long-term administration of TDF causes a gradual decrease in glomerular filtration rate (GFR).[4–7] Furthermore, a growing number of case reports suggested that TDF-associated renal toxicity is mainly caused by proximal tubular injury.[8–11] TDF is excreted via renal proximal tubular transporters.[12,13] Adefovir and cidofovir, both nucleotide analogues, have been reported to cause human renal toxicity via mitochondrial injury in renal tubular epithelial cells.[14] Nevertheless, the impact of the long-term use of TDF on proximal tubular function has not been fully evaluated.

Materials and Methods

This study was conducted prospectively from May 2004 to May 2007. Among 164 HIV-1-infected patients who were registered in Ogikubo Hospital, 110 patients were treated with antiretroviral drugs. Of 110 treated patients, 63 patients who could come to Ogikubo Hospital regularly to have regular blood and urine sampling with informed consents were enrolled in this study. Exclusion criteria were a moderately low level of calculated GFR (<80 ml/min/1.73 m^2). Of 63 enrolled patients, 40 patients were treated with a TDF-based regimen and 23 patients were treated with another NRTI-based regimen. The characteristics of the sample population are shown in Table 1. Of the 40 patients who received TDF, 32 ART-experienced patients simply switched from d4T to TDF to avoid future risk of lipodystrophy or other d4T-related adverse effects. In TDF-receiving patients ($n = 40$), combined NRTIs were as follows: 34 patients with lamivudine (3TC) or emtricitabine (FTC), 4 patients with abacavir (ABC), and 2 patients with didanosine (ddI). In other NRTI-receiving patients ($p = 23$), combinations of two NRTIs were as follows: 10 patients with zidovudine (ZDV) + 3TC, 10 patients with stavudine (d4T) + 3TC, 2 patients with d4T + ddI, and 1 patient with d4T + ABC. Informed consent was obtained from all enrolled patients.

Department of Hematology, Ogikubo Hospital, Tokyo, Japan 167-8515.

TABLE 1. CHARACTERISTICS OF SAMPLE POPULATION

	TDF	*Other NRTI*
Number of patients	40	23
Sex male	40 (100%)	23 (100%)
Median of age (range)	35 (27–66)	32 (22–68)
Median of CD4 (cell/mm^3)	376 (69–1243)	224 (12–748)
Median of HIV RNA (copies/ml)	33 (<50–100,000)	18,000 (<50–100,000)
History of HAART	Naive 8 Experienced 32	Naive 9 Experienced 14
Underlying antiretrovirals		
Efavirenz	14 (35%)	6 (26%)
Nevirapine	3 (8%)	0 (0%)
Atazanavir/ritonavir	16 (40%)	6 (26%)
Lopinavir/ritonavir	5 (13%)	3 (13%)
Nelfinavir	2 (5%)	6 (26%)
Dual therapy	0 (0%)	2 (9%)
Route of HIV-1 infection		
Contaminated blood products	36 (90%)	17 (74%)
Sexual transmission	4 (10%)	6 (26%)
Underlying disease		
Diabetes mellitus	6 (15%)	2 (9%)
Indinavir-associated renal atrophy	2 (5%)	0 (0%)
Pretreatment with indinavir	7 (18%)	7 (0%)

Laboratory testing

Urine β_2-microglobulin (U-BMG), %TRP, alkaline phosphatase (ALP), serum phosphorus, serum uric acid, serum creatinine, and GFR were prospectively measured along with a urinalysis performed every 4–12 weeks, from baseline to 96 weeks, in 40 patients treated with TDF. In 23 patients treated with other NRTIs, serum creatinine, GFR, and ALP were prospectively measured every 3 months, while U-BMG and %TRP were measured every 12 months for 2 years in 17 patients during the same period. U-BMG was determined using a spot urine sample. %TRP was calculated using the following formula: %TRP = [1– (urine phosphorus/serum phosphorus × serum creatinine/urine creatinine)]×100. Urine phosphorus and urine creatinine were measured on the spot urine sample and serum creatinine and serum phosphorus levels were obtained from blood samples on the same day. GFR was calculated based on the simplified modification of diet in renal disease (MDRD) equation, which is described in the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative.[15] In urinalysis, urine glucose and urine protein were evaluated in accordance with the color chart of the dipsticks (Labsticks; Bayer Medical Corp., CA). Renal tubular epithelial cells were counted with high-power fields (HPF; 400× objective), and granular casts were counted with low-power fields (LPF; 100× objective) from urine sediments.

Statistical analyses

Changes over time within groups were assessed using the Wilcoxon signed rank test, and levels of renal parameters between two groups at week 96 were compared using the Mann–Whitney *U* test. Moreover, mixed models analysis of variance (MMANOVA) was used to assess the overall pattern of changes in GFR from baseline to week 96 in the total sample population.[16] MMANOVA allowing for the influence of TDF/other NRTI assignment, naive/experienced assignment, baseline U-BMG level, and a potential interaction between TDF/other NRTI assignment and naive/experienced assignment was applied to adjust the significance level for the change on GFR. All analyses were performed using SAS release 8.02 (SAS Institute, Cary, NC).

Results

Renal parameters

In TDF-receiving patients, 3/40 (8%) patients discontinued TDF before week 96 due to a progressive decline of GFR and tubular dysfunction, whereas there was no patient who discontinued antiretrovirals in other NRTI-receiving patients.

The median [interquartile range (IQR)] of U-BMG was significantly elevated from 188 (134–359) μg/liter at baseline to 555 (229–1425) μg/liter at week 96 ($p = 0.02$) in TDF-receiving patients (Fig. 1a). Highly elevated U-BMG (>10,000 μg/liter) was observed in 3 of 40 (8%) patients by week 96, and moderately elevated U-BMG (1000–10,000 μg/liter) was observed in 15 of 40 (38%) patients by week 96. In contrast, in other NRTI-receiving patients ($n = 17$), U-BMG did not change significantly during the study period [from 170 (96–217) μg/liter at the initial point of the study to 150 (81–307) μg/liter at the end of the study; $p = 0.37$], and there was no patient with a moderate or marked elevation of U-BMG ($\geq$1000 μg/liter).

The median (IQR) of %TRP showed a significant decline from 94 (92–96)% at baseline to 90 (89–95)% at week 96 in TDF-receiving patients ($p = 0.04$; Fig. 1a), whereas there was no significant decline of %TRP in the other NRTI group [$n = 17$; from 96 (95–97)% at the initial point to 94 (91–96)% at week 96 ($p = 0.33$)]. A marked decline in %TRP (<80%) was observed in 3/40 (8%) patients, and a moderate decline (80–90%) was observed in 18/40 (45%) patients at week 96, whereas there was no patient with a moderate or marked decline (%TRP <90%) in the other NRTI group. Comparing the patients who had mildly decreased %TRP (%TRP <90% on two occasions)

with those with normal %TRP (%TRP ≥90%), the former had significantly higher levels of median serum creatinine [0.79 (0.74–0.91) mg/dl vs. 0.63 (0.56–0.70) mg/dl, $p = 0.002$] and significantly lower levels of median GFR [118 (106–136) ml/min/1.73 m^2 vs. 160 (139–177) ml/min/1.73 m^2, $p = 0.001$] at week 96.

The median (IQR) of MDRD-GFR declined gradually from 150 (126–165) ml/min/1.73 m^2 at baseline to 136 (116–157) ml/min/1.73 m^2 at week 96 ($p = 0.02$) in TDF-receiving patients, whereas it did not change in other NRTI-receiving patients ($n = 23$) [from 129 (112–138) ml/min/1.73 m^2 at baseline to 136 (124–145) ml/min/1.73 m^2 at week 96 ($p = 0.39$); Fig. 1b]. In using the Cockcroft-Gault equation, the median (IQR) of GFR also declined from 138 (112–155) ml/min/1.73 m^2 at baseline to 127 (105–148) ml/min/1.73 m^2 at week 96 ($p = 0.02$), whereas it did not significantly change in other NRTI-receiving patients [from 129 (117–143) ml/min/1.73 m^2 at baseline to 135 (116–155) ml/min/1.73 m^2 at week 96 ($p = 0.65$)]. The change in MDRD-GFR over time was reassessed using MMANOVA. In using MMANOVA, GFR declined significantly in TDF-receiving patients (–17 ml/min/1.73 m^2, $p = 0.04$), whereas it did not change in other NRTI-receiving patients (+3 ml/min/1.73 m^2, $p = 0.43$). The overall difference between the two treatment group was statistically significant (MMANOVA, $p = 0.03$). GFR change was not significantly influenced by previous administration of HAART (MMANOVA, $p = 0.07$) or baseline U-BMG levels (MMANOVA, $p = 0.28$). There was no significant interaction between TDF/other NRTI assignment and naive/experience assignment (MMAOVA, $p = 0.73$). The median (IQR) of serum creatinine increased from 0.64 (0.59–0.75) mg/dl at baseline to 0.74 (0.64–0.80) mg/dl at week 96 ($p = 0.02$), whereas it did not change in other NRTI-receiving patients [$n = 23$; from 0.73 (0.68–0.83) mg/dl at baseline to 0.70 (0.66–0.78) mg/dl at week 96 ($p = 0.14$), respectively].

The median (IQR) of ALP persistently and significantly rose during the study period in the TDF group [from 289 (261–382) IU/liter at baseline to 355 (280–421) IU/liter at week 96 ($p = 0.001$)], whereas it did not change significantly in other NRTI groups [from 172 (138–250) IU/liter at baseline to 180 (148–247) IU/liter at week 96 ($p = 0.98$)]. In comparing the ALP ratio (relative to baseline), the median (IQR) ALP ratio in patients receiving TDF was significantly higher than in patients receiving other NRTIs [1.278 (1.059–1.354) vs. 1.003 (0.876–1.098) ($p = 0.02$); Fig. 1c]. Even in patients receiving TDF, serum phosphorus and serum uric acid were not significantly decreased during the study period. The median (IQR) serum phosphorus level was 3.4 (2.9–3.6) mg/dl at baseline and 3.0 (2.7–3.4) mg/dl at week 96 ($p = 0.20$), and serum uric acid was 6.1 (5.0–7.0) mg/dl at baseline and 5.5 (4.9–6.6) mg/dl at week 96 ($p = 0.08$).

In TDF-receiving patients, a reduction in GFR level was associated with U-BMG levels. GFR significantly decreased in patients with higher U-BMG (≥1000 μg/liter) in two or more occasions from 132 (124–159) ml/min/1.73 m^2 at baseline to 118 (104–151) ml/min/1.73 m^2 at week 96 ($p = 0.01$), whereas it did not decrease in the other patients [from 155 (134–172) mg/dl at baseline to 143 (133–164) mg/dl ($p = 0.59$)]. In comparing GFR levels at week 96 between the patients with higher U-BMG (≥1000 μg/liter) on two or more occasions and those with lower U-BMG (<1000 μg/liter), the former level was significantly lower than the latter [118 (104–151) ml/min/1.73 m^2 vs. 143 (133–164) ml/min/1.73 m^2 ($p = 0.04$)].

In urinalysis, the ratio of the patients with positive urine protein did not significantly increase in both TDF-receiving patients and other NRTI-receiving patients [19% at baseline and 26% at week 96 ($p = 0.84$) in TDF-receiving patients and 5% at baseline and 5% at week 96 in other NRTI-receiving patients ($p = 0.86$), respectively]. There was no statistical difference in the ratio of positive urine protein at week 96 between the two groups. A ratio of the patients with positive urine glucose did not significantly change in both TDF-receiving patients and other NRTI-receiving patients [19% at baseline and 26% at week 96 in TDF-receiving patients ($p = 0.84$) and 5% at baseline and 5% at week 96 in other NRTI-receiving patients, respectively]. Granular cast was observed in 5% at baseline and 6% at week 96 in TDF-receiving patients, and 0% at baseline and 0% at week 96 in other NRTI-receiving patients. There was no significant difference between the two groups at baseline or week 96 ($p = 0.77$ and 0.85, respectively). Renal tubular epithelial cells were observed in 17% at baseline and 8% at week 96 in TDF-receiving patients ($p = 0.46$), and 5% at baseline and 9% at week 96 in other NRTI-receiving patients. There was no significant difference between the two groups at baseline or week 96 ($p = 0.29$ and 0.43, respectively). Among the five TDF-receiving patients with rapid deterioration of U-BMG and %TRP, granular casts were observed in only two patients and renal tubular epithelial cells were observed in three patients.

Severe TDF-associated renal toxicity and its recovery after discontinuation of TDF

In this study, severe renal toxicity was observed in five TDF-receiving patients (Table 2), whereas neither reduction of GFR nor tubular dysfunction was observed in other NRTI-receiving patients. Among these five patients, three patients (Patients 1–3 in Table 2) showed TDF-associated renal toxicity during the study period, and they discontinued TDF. The other two patients had acute renal failure after the study period (Patients 4 and 5). An extremely abnormal value of U-BMG (>10,000 μg/liter) and %TRP (<80%) were observed in all five patients, but both of them recovered to baseline levels immediately after TDF was discontinued in all cases. In three of five patients, GFR levels rapidly declined from a normal level (>90 ml/min/1.73 m^2) to a mildly decreased level (60–89 ml/min/1.73 m^2), and in the other two patients, it declined from normal to a moderately decreased level (30–59 ml/min/1.73 m^2). In three patients (Patient 1, 2, and 4), the GFR level did not fully recover for 6 months after discontinuation of TDF (Table 2).

No association between TDF-associated renal toxicity and low CD4 cell count

Among TDF-receiving patients, urine-β_2-microglobulin, %TRP, ALP, GFR, and serum creatinine were compared between patients with low CD4 cell counts at baseline (<200/μl; $n = 11$) and patients with normal CD4 cell counts at baseline (≥200/μl; $n = 29$). In the 11 patients with low CD4 cell count <200, U-BMG at baseline and week 96 was 307 (235–455) μg/liter and 411 (262–711) μg/liter; %TRP was 94

(92–95)% and 90 (88–93)%; GFR was 159 (146–174) ml/min/1.73 m^2 and 147 (134–163) ml/min/1.73 m^2; and serum creatinine was 0.62 (0.57–0.66) mg/dl and 0,67 (0.62–0.74) mg/dl. In contrast, in the 29 patients with normal CD4 cell count >200, U-BMG at baseline and week 96 was 154 (113–194) μg/liter and 499 (208–1790) μg/liter; %TRP was 94 (92–96)% and 91 (85–93)%; GFR was 151 (130–163) mlmin/1.73 m^2 and 130 (116–157) ml/min/1.73 m^2; and serum creatinine was 0.64 (0.59–0.75) mg/dl and 0.74 (0.64–0.80) mg/dl.

Discussion

Several large clinical studies revealed that long-term use of TDF caused a gradual reduction of GFR, whereas tubular dysfunction has not been fully evaluated. Although increased urinary loss of BMG has already been observed in the patients treated with TDF both in adults[17] and children,[18,19] it has not been determined whether long-term use of TDF causes tubular dysfunction, or whether TDF-associated tubular dysfunction is persistent/progressive or transient. This study first showed that long-term use of TDF caused progressive tubular dysfunction, whereas a decline in GFR was significant but minimal. Three of five patients with severe proximal tubular dysfunction (U-BMG > 10,000 μg/liter and %TRP < 80%) did not show a marked low level of estimated GFR (<60 ml/min/1.73 m^2), although they presented with a rapid reduction of estimated GFR. Also, in a previously reported case with typical Fanconi's syndrome,[20] maximum serum creatinine was 1.06 mg/dl and minimum calculated GFR was 82 ml/min/1.73 m^2. Measuring tubular function is useful to detect progressive tubular dysfunction, which causes Fanconi's syndrome.

Some analyses suggested a potential association of progressive tubular dysfunction and a gradual decline of GFR in TDF-associated renal impairment. In TDF-receiving patients, GFR levels in patients with high U-BMG (≥1000 μg/liter) on two or more occasion were significantly lower than that in patients with lower U-BMG (<1000 μg/liter). In analyses using MMANOVA for the total sample population, maximum U-BMG levels were significantly associated with the

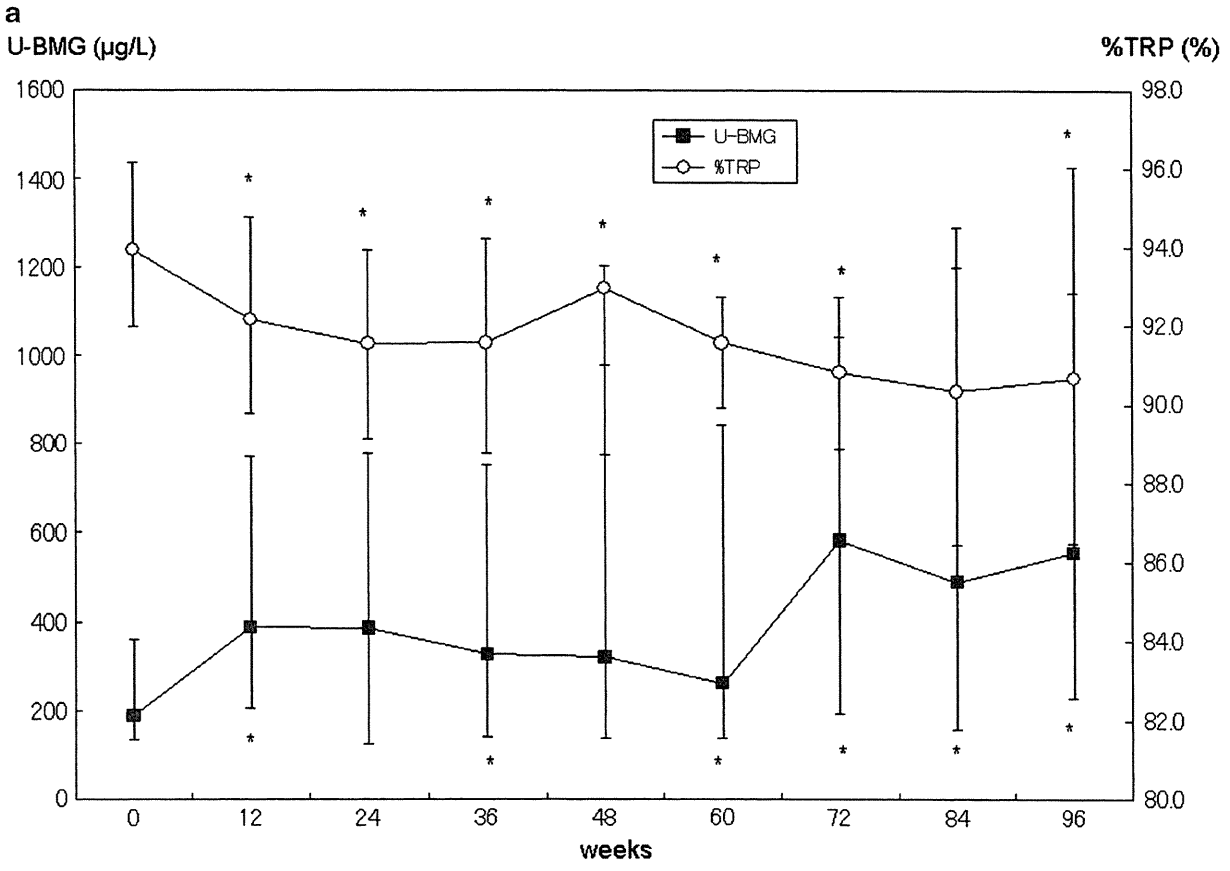
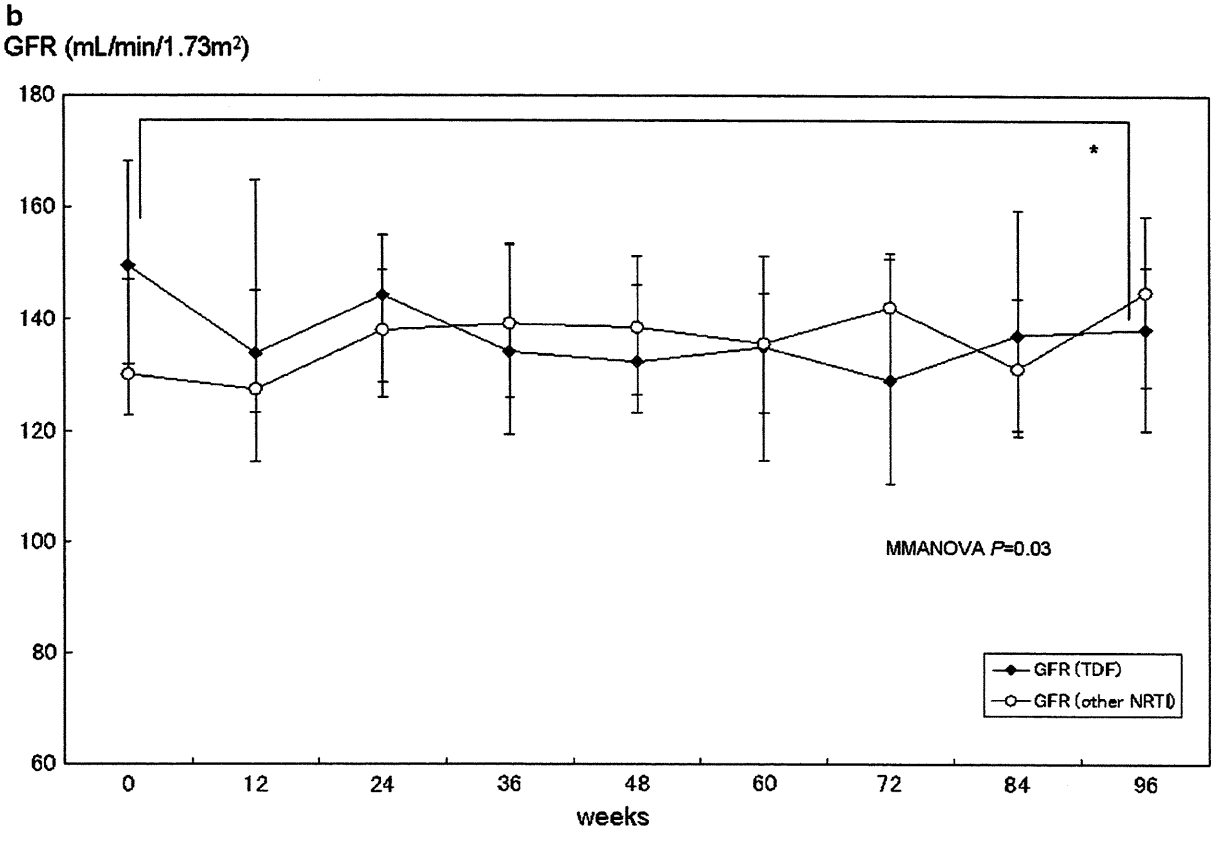


FIG. 1. Urine β_2-microglobulin (U-BMG) and percentage tubular reabsorption of phosphate (%TRP), calculated glomerular filtration rate (GFR), and alkaline phosphatase. (**a**) The U-BMG level in patients receiving TDF (■) and %TRP in patients receiving TDF (○). (**b**) The glomerular filtration rate (GFR) in patients receiving TDF (■) and in patients receiving other NRTIs (○). GFR was calculated using the simplified modification of diet in renal disease (MDRD) formula. (**c**) The ratio of alkaline phosphatase compared with baseline in patients receiving TDF (■) and patients receiving other NRTIs (○). Data are shown as median (IQR). $^*p < 0.05$, $^{**}p < 0.001$ using the Wilcoxon signed rank test.



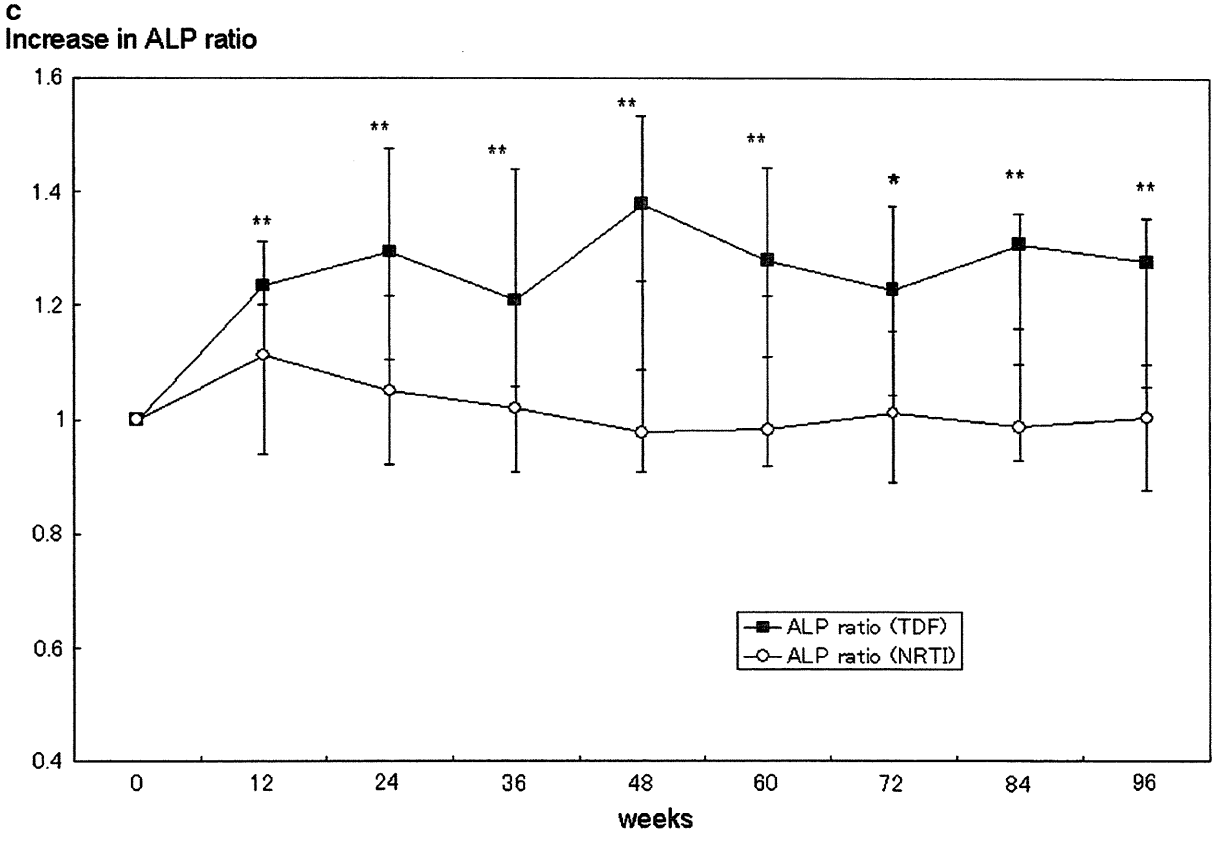


FIG. 1. (*Continued*).

TABLE 2. COMPARISON OF GFR, SERUM CREATININE, URINE-β_2MG, AND TRP(%) AT BASELINE, WORST LEVEL DURING TDF-RECEIVING, AND RECOVERY LEVEL AFTER DISCONTINUATION OF TDF IN FIVE PATIENTS WITH SEVERE RENAL TOXICITY[a]

Patient No.	Key drug	TDF duration (weeks)	Risk factors	GFR (ml/min/1.73 m^2)			Urine-β_2MG (μg/liter)			TRP (%)		
				BL	Minimum	Recovery	BL	Maximum	Recovery	BL	Minimum	Recovery
1	NNRTI	12	DM	101	62	82	406	55,100	329	87	42	91
2	NNRTI	12	DM	92	45	65	354	79,900	1150	78	19	79
3	PI	1	IDV-related renal atrophy	124	108	102	86	49,900	110	99	74	89
4	PI	106	DM, IDV-related renal atrophy, VCM, NSAIDs	126	48	68	260	22,800	537	96	50	85
5	NNRTI	194	TMP-SMX	159	70	109	711	11500	984	93	52	82

[a]BL, baseline; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; DM, diabetes mellitus; IDV, indinavir; TMP-SMX, trimethoprim-sulfamethoxazole; VCM; vancomycin; NSAIDs: nonsteroidal anti-inflammatory drugs.

GFR level at week 96 (MMANOVA, $p = 0.0002$). Additionally, in 12/40 (30%) TDF-receiving patients with a moderate decrease in GFR (<70% of baseline) by week 96, a significantly higher level of U-BMG [2750 (2050–12800) μg/liter vs. 263 (185–578) μg/liter at week 96, $p = 0.008$] and a relatively lower %TRP [88 (84–91)% vs. 91 (86–95)% at week 96, $p = 0.15$] were observed. Of interest, among these 12 patients, elevated U-BMG (maximum U-BMG >1000 μg/liter) was observed in 56% by week 12, in 67% by week 24, and in 100% at week 48, whereas the decrease in GFR (minimum GFR <70% of baseline) was observed in only 18% of patients by week 12, in 36% by week 24, and in 55% by week 48. Early elevation of U-BMG (>1000 μg/liter) seemed to be one of the useful predictors of a modest decline in GFR. However, it could not be statistically determined which level of U-BMG and which week of measurement would be the best predictors of a decline in GFR.

It has not been determined yet whether TDF-associated renal dysfunction is reversible. Some case reports also suggested that rapidly deteriorating renal function can result in irreversible or fatal renal failure.[8–11] In this study, the GFR did not fully recover for 6 months in three of five patients. In a case with severe renal dysfunction, a longer observation period (>6 months) may be required to determine whether the GFR truly recovers. Moreover, all of the above five patients had some risk factors for renal impairment, such as diabetes mellitus or IDV-associated renal atrophy, although their GFR levels at baseline were above 90 ml/min/1.73 m^2. Therefore, it is still uncertain whether the GFR level will recover in the patients without risk factors after discontinuing TDF. In contrast, and importantly, quite severe tubular dysfunction immediately recovered to the baseline level after discontinuing TDF in all of the five cases and in the previously reported one case[20] with severe renal toxicity, regardless of any risk factors. It may be safer to decide to continue or discontinue TDF in accordance with the U-BMG level or %TRP level, if possible.

This study was conducted prospectively and available patients at Ogikubo Hospital were enrolled without any selection. Although the number of patients in each sample group was small and different ($n = 40$ in TDF-receiving patients vs. $n = 23$ in other NRTI-receiving patients) due to a small available sample population, there was no significant difference in patient characteristics between TDF-receiving patients and NRTI-receiving patients. Moreover, there were more risk factors for renal impairment in those receiving other NRTIs—the median CD4 cell count was lower, the median HIV RNA was higher, and the percentage of the patients pretreated with IDV was higher.

Despite these findings, none of the other NRTI-receiving patients showed deterioration of any renal parameters. Additionally, characteristics of the sample population did not greatly deviate from those of the general population, since the change of renal function of TDF-receiving patients in this study was consistent with other large observational studies. The ratio of the elevation in the serum creatinine level is comparable to other observational studies.[21,22]

On the other hand, it is uncertain whether the observed overall change in GFR in this study was applicable to general populations. The absolute decline in GFR by −17 ml/min/1.73 m^2 in and the proportion of 12/40 (30%) patients with a modest decrease in GFR (<70% of baseline) in TDF-receiving patients after 2 years was comparable to large observational studies.[4,23] However, another observational study showed a lower ratio of the decline in GFR.[5] This may provide a limitation to the generalization of this conclusion.

There are some limitations to the use of U-BMG testing in the routine monitoring of TDF-treated patients. Although the U-BMG level is a specific marker of proximal tubular dysfunction, it sometimes becomes elevated in progressive HIV infection and often varies according to serum β_2-microglobulin level.[24] However, because markedly elevated U-BMG (>10,000 μg/liter) is quite rare even in progressive HIV infection,[24] the criterion of discontinuing TDF (10,000 μg/liter) may be reasonable.

The percentage TRP, which directly reflects the urinary loss of phosphorus, has been shown to be quite sensitive in detecting tubular dysfunction. ALP, a marker of osteoblastic activity, showed persistent increases in patients receiving TDF, whereas the serum phosphorus level did not decrease. Tubular dysfunction does not cause an immediate decrease in serum phosphorus levels because the level is maintained by bone mineralization.[24] An indicator of urinary loss of phosphorus is not the serum phosphorus level but %TRP and ALP level. However, the ALP level can be affected by other factors including liver disease, therefore, an increased ALP ratio compared with baseline is a better measure of bone mineralization.

Urinalysis is not only a simple and low-cost test but is also useful for detecting severe renal injury; therefore the Infectious Diseases Society of America recommended urinalysis as a screening test for HIV-related renal disease.[15] However, urinalysis using a dipstick cannot detect urinary loss of low-molecular-weight protein, which is a typical finding in TDF-related tubular toxicity.[25] Although glucosuria can be observed in severe Fanconi's syndrome, it was not detected in mild tubular dysfunction. Renal tubular epithelial cells and granular casts are generally considered as a sensitive and specific marker for acute tubular necrosis. However, in this study, they were observed only three of five of the patients with severe TDF-associated renal toxicity. Additionally, they were sometimes observed nonspecifically in patients without acute or chronic renal dysfunction.

Progression of HIV infection causes chronic renal dysfunction, and a very low CD4 cell count (<50/μl) was reported to be a risk factor for TDF-associated renal toxicity.[4] However, this study suggested that a low CD4 cell count was not a risk factor for acute nephrotoxicity. A very low CD4 cell count (<50/μl) may be more frequently associated with renal damage than a moderately low CD4 cell count (<200/μl) [26,27]; in addition, nephrotoxic agents are frequently used in patients with very low CD4 cell counts, for example, NSAIDs, TMP-SMX, and amphotericin B.

In conclusion, prolonged treatment with TDF caused progressive proximal tubular dysfunction as well as a modest decline in GFR. A critical threshold of U-BMG or %TRP for discontinuing TDF was not determined, because this study was not designed to determine criteria guiding either the use of tenofovir or its discontinuation. However, if apparently abnormal tubular dysfunction, such as a persistent elevation of U-BMG >10,000 μg/liter or a decline of %TRP (<80%), is observed, closer monitoring of GFR is necessary, and if possible, discontinuing TDF may be beneficial.

Disclosure Statement

No competing financial interests exist.

References

1. Schooly RT, Ruane P, Myers RA, *et al.*, for the Study 902 Team: Tenofovir DF in antiretroviral-experienced patients: Results from a 48-week, randomized, double-blind study. AIDS 2002;16:1257–1263.
2. Gallant JE, Staszewski S, Pozniak AL, *et al.*, for the 903 Study Group: Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naïve patients. JAMA 2004;292:191–201.
3. Gallant JE, DeJesus E, Arribas JR, *et al.*, for the Study 934 Group: Tenofovir DF, emtricitabine, and efavirenz vs. zidovudine, lamivudine, and efavirenz for HIV. N Engl J Med 2006;354:251–260.
4. Gallant JE, Parish MA, Keruly JC, *et al.*: Changes in renal function associated with tenofovir disoproxil fumarate treatment, comapred with nucleoside reverse-transcriptase inhibitor treatment. Clin Infect Dis 2005;40:1194–1198.
5. Fux CA, Simcock M, Wolbers M, *et al.*, for the Swiss HIV Cohort Study: Tenofovir use is associated with a reduction in calculated glomerular filtration rates in the Swiss HIV Cohort Study. Antiviral Ther 2007;12:1165–1173.
6. Mocroft A, Kirk O, Gatell J, *et al.*, for the EuroSIDA Study Group: Chronic renal failure among HIV-1-infected patients. AIDS 2007;21:1119–1127.
7. Julg BD, Bogner JR, Crispin A, *et al.*: Progression of renal impairment under therapy with tenofovir. AIDS 2005;19(12): 1332.
8. Verhelst D, Monge M, Meynard JL, *et al.*: Fanconi syndrome and renal failure induced by tenofovir: A first case report. Am J Kidney Dis 2002;40:1331–1333.
9. Karras A, Lafaurie M, Furco A, *et al.*: Tenofovir-related nephrotoxicity in human immunodeficiency virus-infected patients: Three cases of renal failure, Fanconi syndrome, and nephrogenic diabetes insipidus. Clin Infect Dis 2003;36: 1070–1073.
10. Peyriere H, Reynes J Rouanet I, Daniel N, de Boever, CM, Mauboussin JM, Leray H, Moachon L, Vincent D, and Salmon-Ceron D: Renal tubular dysfunction associated with tenofovir therapy: Report of 7 cases. J Acquir Immune Defic Syndr 2004;35:269–273.
11. Zimmerman AE, Pizzoferrato T, Bedford J, Morris A, Hoffman, R, and Braden G: Tenofovir-associated acute and chronic kidney disease: A case of multiple drug interactions. Clin Infect Dis 2006;40:283–290.
12. Cihlar T, Ho ES, Lin DC, and Mulato AS: Human renal organic anion transporter 1 (hOAT1) and its role in the nephrotoxicity of antiviral nucleotide analogs. Nucleoside Nucleotides Nucleic Acids 2001;20:641–648.
13. Izzedine H, Launay-Vacher V, and Deray G: Antiviral drug-induced nephrotoxicity. Am J Kidney Dis 2005;45:804–817.
14. Tanji N, Tanji K, Kambham N, Markowitz GS, Bell A, and D'aqati VD: Adefovir nephrotoxicity: Possible role of mitochondrial DNA depletion. Hum Pathol 2001;32:734–740.
15. Gupta SK, Eustace JA, Winston JA, Boydstun II, Ahuja TS, Rodriguez RA, Tashima KT, Roland M, Franceschini, N, Palella FJ, Lennox JL, Klotman PE, Nachman, SA, Hall SD, and Szczech LA: Guidelines for the management of chronic kidney disease in HIV-infected patients: Recommendations of the HIV Medicine Association of the Infectious Diseases Society of America. Clin Infect Dis 2005;40:1559–1585.
16. Dube MP, Parker RA, Tebas P, Grinspoon SK, Zackin RA, Robbins GK, Roubenoff R, Shafer RW, Wininger DA, Meyer WA III, Snyder SW, and Mulligna K: Glucose metabolism, lipid, and body fat chagnes in antiretroviral-naive subfects randomized to nelfinavir or efavirenz plus dual nucleosides. AIDS 2005;19:1807–1818.
17. Gatanaga H, Tachikawa N, Kikuchi Y, Teruya K, Genka I, Honda M, Tanuma J, Yazaki H, Ueda A, Kimura S, and Oka S: Urinary β_2-microglobulin as a possible sensitive marker for renal injury caused by tenofovir disoproxil fumarate. AIDS Res Hum Retroviruses 2006;22(8):744–748.
18. Papaleo A, Warszawski J, Salomon R, Jullien V, Veber F, Dechaux M, and Blanche S: Increased β-2 microglobulinuria in human immunodeficiency virus-1-infected children and adolescents treated with tenofrovir. Pediatr Infect Dis J 2007; 26(10):949–951.
19. Vigano A, Zuccotti GV, Martelli L, Giacomet V, Cafarelli L, Borgonovo S, Beretta S, Rombola G, and Mora S: Renal safety of tenofovir in HIV-infected children: A prospective, 96-week longitudinal study. Clin Drug Investig 2007;27(8):573–581.
20. Clinical practice guidelines of the Kidney Disease Outcomes Quality Initiative of the National Kidney Foundation. Available at http://www.kidney.org/professionals/kdoqi/guidelines.cfm.

21. Kinai E and Hanabusa H: Renal tubular toxicity associated with tenofovir assessed using urine-beta 2 microglobulin, percentage of tubular reabsorption of phosphate and alkaline phosphatase levels. AIDS. 2005;19(17):2031–2033.
22. Nelson MR, Katlama C, Montaner JS, Cooper DA, Gazzard B, Clotet B, Lazzarin A, Schewe K, Lange J, Wyatt C, Curtis S, Chen SS, Smith S, Bischofberger N, and Rooney JF, for the Tenofovir DF Expanded Access Team: The safety of tenofovir disoproxil fumarate for the treatment of HIV infection in adults: The first 4 years. AIDS 2007;21(10):1273–1281.
23. Gerard L, Chazallon C, Taburet AM, Girard PM, Aboulker JP, and Piketty C: Renal function in antiretroviral-experienced patients treated with tenofovir disoproxil fumarate associated with atazanavir/ritonavir. Antiviral Ther 2007;12:31–39.
24. Moore RD and Gallant JE: Renal function after use of tenofovir as part of the initial ART regimen. 48th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, October 25–28, 2008.
25. Kabanda A, Vandercam B, Bernard A, Lauwerys R, and van Ypersele de Strihou C: Low molecular weight proteinuria in human immunodeficiency virus-infected patients. Am J Kidney Dis 1996;27:803–808.
26. Earle KE, Seneviratne T, Shaker J, and Shoback D: Fanconi's syndrome in HIV^{+} adults: Report of three cases and literature review. J Bone Miner Res 2004;19:714–721.
27. Kimmel PL, Barisoni L, and Kopp JB: Pathogenesis and treatment of HIV-associated renal diseases: Lessons from clinical and animal studies, molecular pathologic correlations, and genetic investigations. Ann Intern Med 2003;139: 214–226.
28. Röling J, Schmid H, Fischereder M, Draenert R, and Goebel FD: HIV-associated renal diseases and highly active antiretroviral therapy-induced nephropathy. Clin Infec Dis 2006; 42:1488–1495.

Address reprint requests to:
Ei Kinai
Department of Hematology
Ogikubo Hospital
Imagawa 3-1-24
Suginami-ku
Tokyo, Japan 167-8515

E-mail: ekinai@mui.biglobe.ne.jp

Australian and New Zealand Journal of Obstetrics and Gynaecology 2009; **49:** 137–141 DOI: 10.1111/j.1479-828X.2009.00958.x

Original Article

Antibody responses to *Porphyromonas gingivalis* outer membrane protein in the first trimester

Jun SASAHARA,[1] Akira KIKUCHI,[1] Koichi TAKAKUWA,[1] Noriko SUGITA,[2] Yoshimitsu ABIKO,[3] Hiromasa YOSHIE[2] and Kenichi TANAKA[1]

[1]*Department of Obstetrics and Gynecology, Niigata University Graduate School of Medical and Dental Sciences, Niigata,* [2]*Division of Periodontology, Department of Oral Biological Science, Niigata University Graduate School of Medical and Dental Sciences, Niigata, and* [3]*Department of Biochemistry and Research Institute of Oral Science, Nihon University School of Dentistry, Matsudo, Chiba, Japan*

Background: *Porphyromonas gingivalis* (*Pg*) is one of the most harmful periodontal pathogens and it has been reported that *Pg* is associated with preterm birth (PTB), intrauterine growth retardation (IUGR) and pregnancy-induced hypertension (PIH), discovered by animal experiments and clinical research. The relationship between adverse pregnancy outcomes and maternal antibody response to *Pg* is controversial. On the other hand, the serum C-reactive protein (CRP) has been recognised as a reliable serum marker of periodontal disease.

Aims: To determine the significance of antibody responses to *Pg* affecting pregnancy outcomes in the first trimester.

Methods: A case–control study was carried out on women with PTB ($n = 58$), IUGR ($n = 91$), PIH ($n = 32$) and without any complications (control, $n = 98$). The serum level of the CRP and IgG1 against 40-kDa outer membrane protein of *Pg* (anti-40-kDa OMP *Pg*-IgG1) in the first trimester was measured.

Results: The IUGR group, and PTB patients whose placentas were diagnosed as chorioamnionitis or whose vaginal flora included *Lactobacilli*, showed a lower level of anti-40-kDa OMP *Pg*-IgG1 than the control group. There was no difference in the serum CRP level between each case group and control group.

Conclusions: These results suggest that a lack of humoral immunity against *Pg* in early pregnancy is associated with IUGR and some PTB.

Key words: intrauterine fetal growth retardation, periodontal disease, *Porphyromonas gingivalis*, pregnancy-induced hypertension, preterm birth.

Introduction

Periodontal disease is chronic infection of tooth-supporting tissues, which is caused by oral bacteria. For decades, many studies have indicated that periodontal disease is not only a problem of the oral cavity but also is very much related to systemic diseases, such as cardiovascular disease, type 2 diabetes mellitus, osteoporosis and so on.[1] In the obstetrical field, it is suggested that preterm birth (PTB), low birthweight, intrauterine growth retardation (IUGR), pre-eclampsia and miscarriage are associated with periodontal disease, and the mechanisms may be host-immune responses against oral bacterial infection or a direct bacterial assault on the placenta and fetus.[1–3] *Porphyromonas gingivalis* (*Pg*) is one of the most important periodontal pathogens and it is strongly suggested that *Pg* could cause adverse pregnancy outcomes, discovered by animal experiments and clinical research.[4–7] The outer membrane protein (OMP) of *Pg* is a major virulence factor associated with bacteria colonisation in the gingival crevice. Chronic periodontitis patients showed a significantly higher serum level of IgG, especially the IgG1 subclass, against 40-kDa OMP of *Pg* (anti-40-kDa OMP *Pg*-IgG1).[8] The purpose of this study was to determine the relation between pregnancy outcomes and the serum level of anti-40-kDa OMP *Pg* -IgG1 in the first trimester.

Methods

The subjects were selected from Japanese pregnant women who underwent blood sampling during the first trimester between January 1994 and May 2002 and delivered at Niigata University Medical and Dental Hospital. The Ethics Committee of Niigata University approved this study. One hundred and eighty-one women with adverse pregnancy outcomes and 98 control women were enrolled in this study.

The adverse pregnancy outcomes indicated the causes to be PTB, IUGR and pregnancy-induced hypertension (PIH).

Correspondence: Dr Akira Kikuchi, Department of Obstetrics and Gynecology, Niigata University Graduate School of Medical and Dental Sciences, 1-754, Asahimachi-dori, Niigata City, Niigata 951-8510, Japan. Email: no4achy@med.niigata-u.ac.jp

Received 16 June 2008; accepted 11 October 2008.

Table 1 Clinical characteristics of each group

	Maternal age, years median (IQR)	Primipara, %	Gestational age, weeks median (IQR)	Birthweight, grams median (IQR)
PTB ($n = 58$)	33.0 (29.3, 36.8)	44.8	35.9 (34.6, 36.4)*	2230 (1828, 2542)*
IUGR ($n = 91$)	33.0 (29.0, 36.0)†	54.1	39.4 (38.6, 40.2)†	2504 (2376, 2639)*
PIH ($n = 32$)	33.0 (29.8, 36.0)†	37.5	39.0 (36.4, 40.5)†	2729 (2043, 3156)*
Control ($n = 98$)	31.5 (28.0, 34.0)	57.1	39.9 (39.0, 40.4)	3068 (2911, 3352)

P-values were determined by Mann–Whitney *U*-test or Fisher exact test. *, $P < 0.01$; †, $P < 0.05$.
CRP, C-reactive protein; IQR, interquartile range; IUGR, intrauterine growth retardation; PIH, pregnancy-induced hypertension; PTB, preterm birth.

PTB is defined as delivery at less than 37 weeks of gestation, as a result of either preterm labour or premature rupture of the membranes (PROM). IUGR is defined as delivery of an infant whose birthweight is less than the 10th percentile of Japanese standards. According to the criteria of the Japanese Society of Obstetrics and Gynecology (2005), PIH is defined as hypertension with or without proteinuria occurring after the 20th week of gestation but being resolved by the 12th post-partum week. It encompasses pre-eclampsia (hypertension plus proteinuria), gestational hypertension (hypertension without proteinuria) and superimposed pre-eclampsia. Superimposed pre-eclampsia is defined as the new onset of proteinuria after the 20th week of gestation in a woman with chronic hypertension and no proteinuria before the 20th week of gestation, or a sudden increase in blood pressure and/or proteinuria after the 20th week of gestation in a woman with both hypertension and proteinuira before the 20th week of gestation, or the new onset of hypertension after the 20th week of gestation in a woman with proteinuria and no hypertension before the 20th week of gestation. Mild PIH was diagnosed where there was systolic blood pressure higher than 140 mmHg but not exceeding 160 mmHg and/or diastolic blood pressure higher than 90 mmHg but not exceeding 110 mmHg, as well as daily proteinuria higher than 300 mg but not exceeding 2 g. Severe PIH was diagnosed where there was systolic blood pressure exceeding 160 mmHg and/or diastolic blood pressure exceeding 110 mmHg, as well as daily proteinuria exceeding 2 g. PIH with an onset earlier than the 32nd week of gestation is defined as being early onset, and PIH with an onset after the 32nd week is defined as being late onset. Patients who experienced PIH and delivered IUGR infants, were classified in the PIH group, and those who delivered IUGR infants as a result of preterm labour or PROM, were classified in the PTB group. The control group consisted of women who delivered appropriate birthweight infants after 37 weeks of gestation without pregnancy-induced hypertension (PIH) or other medical problems.

In the case of PTB patients, vaginal swabs were obtained for bacterial culture tests when they were admitted with preterm labour or PROM, and their placentas were examined for histological chorioamnionitis (CAM) after delivery. CAM was diagnosed if there was acute inflammation with polymorphonuclear leucocytes infiltrating the chorionic membrane. Exclusion criteria included multiple birth, congenital fetal abnormalities and incompetent cervix.

Peripheral venous blood samples were obtained from first trimester women. Serum was collected by centrifugation at 1500 *g* for 20 min and stored at – 20°C until use. Recombinant 40-kDa OMP of *Pg* was purified by the method of Kawamoto *et al.* and anti-40-kDa OMP *Pg*-IgG1 was determined by enzyme-linked immunosorbent assay, as previously described.[8,9] Antibody levels were expressed as percentages of the control serum. The control serum was derived from a patient with periodontitis and with *Pg* detected in her periodontal pockets by polymerase chain reaction (PCR). A CRP assay was performed by Latex Photometric Immunoassay on QUICK TURBO C (SHINO-TEST, Tokyo, Japan).

The statistical differences from the control group were determined by the Mann–Whitney *U*-test or Fisher exact test. All analyses were performed using the R version 2.6.1. and R Commander version 1.3-9.

Results

A total of 279 subjects (58 PTB, 91 IUGR, 32 PIH and 98 controls) were enrolled in the study. The clinical characteristics of each group are shown in Table 1. There were no differences in the percentage of primipara between each case group and the control group. The maternal ages of those in IUGR and PIH groups were significantly higher than those of the control group. As expected, the PTB, IUGR and PIH groups had significantly lower gestational ages and birthweights than the control group. Anti-40-kDa OMP *Pg*-IgG1 of the IUGR group was significantly lower than that of the control group and no differences were observed among the PTB, PIH and control groups (Table 2). However, PTB patients whose placentas were diagnosed as CAM showed a lower tendency ($P = 0.055$), and those who were *Lactobacillus*-positive in vaginal flora showed significantly lower levels than the control group (Table 3). Although there were eight PTB patients who suffered from CAM despite the presence of *Lactobacilli*, they showed very low serum levels of anti-40-kDa OMP *Pg*-IgG1 (median 14.5%, interquatile range: 6.7~37.4%). As shown in Table 4, there were no differences in anti-40-kDa OMP*Pg*-IgG1 between the control group and each subclass of PIH (gestational hypertension, pre-eclampsia, superimposed pre-eclampsia, early onset, late onset, mild

Table 2 Serum level of anti 40-kDa *Pg*-IgG1 and CRP in the first trimester

	Anti 40-kDa *Pg*-IgG1, %		CRP, mg/dL	
	Median (IQR)	*P*-value	Median (IQR)	*P*-value
PTB ($n = 58$)	80.4 (35.0, 166.5)	0.226	0.12 (0.03, 0.28)	0.115
IUGR ($n = 91$)	57.3 (29.4, 151.4)	< 0.01	0.06 (0.02, 0.18)	0.684
PIH ($n = 32$)	82.8 (48.1, 90.7)	0.118	0.06 (0.20, 0.19)	0.315
Control ($n = 98$)	100.3 (54.6, 177.6)		0.06 (0.03, 0.19)	

P-values were determined by Mann–Whitney *U*-test.
CRP, C-reactive protein; IQR, interquartile range; IUGR, intrauterine growth retardation; PIH, pregnancy-induced hypertension; PTB, preterm birth.

Table 3 Relation of CAM and *Lactobacilli* with anti-40-kDa *Pg*-IgG1 and CRP in preterm birth group

		Anti-40-kDa *Pg*-IgG1, %		CRP, mg/dL	
	n	Median (IQR)	*P*-value	Median (IQR)	*P*-value
CAM					
Positive	24	52.6 (22.1, 143.3)	0.055	0.11 (0.05, 0.27)	0.098
Negative (unknown 13)	21	136.7 (63.2, 210.0)	0.334	0.18 (0.02, 0.44)	0.109
Lactobacilli in vaginal flora					
Positive	22	38.1 (8.5, 127.7)	< 0.01	0.08 (0.02, 0.24)	0.849
Negative (unknown 20)	16	144.8 (66.7, 226.3)	0.114	0.20 (0.05, 0.35)	0.077
Control	98	100.3 (54.6, 177.6)		0.06 (0.03, 0.19)	

P-values were determined using Mann–Whitney *U*-test.
CAM; histological chorioamnionitis; CRP; C-reactive protein; IQR, interquatile range.

Table 4 Anti-40-kDa *Pg*-IgG1, CRP and subclasses of pregnancy-induced hypertension

		Anti-40-kDa *Pg*-IgG1, %		CRP, mg/dL	
	n	Median (IQR)	*P*-value	Median (IQR)	*P*-value
Gestational hypertension	9	71.1 (37.3, 85.2)	0.146	0.05 (0.00, 0.08)	0.194
Pre-eclampsia	15	83.6 (67.9, 88.1)	0.206	0.03 (0.02, 0.14)	0.308
Superimposed pre-eclampsia	8	90.4 (67.4, 161.6)	0.910	0.14 (0.04, 0.26)	0.549
Early onset type	11	81.6 (54.6, 120.7)	0.590	0.08 (0.03, 0.22)	0.661
Late onset type	21	83.6 (49.8, 88.9)	0.101	0.03 (0.00, 0.08)	0.108
Mild	18	83.1 (44.7, 90.1)	0.104	0.04 (0.00, 0.08)	0.106
Severe	14	82.6 (73.7, 90.7)	0.507	0.07 (0.02, 0.24)	0.826
Control	98	100.3 (54.6, 177.6)		0.06 (0.03, 0.19)	

P-values were determined using Mann–Whitney *U*-test.
CRP, C-reactive protein; IQR, interquatile range.

and severe PIH). There was also no difference in the serum CRP level between the control group and each case group (Tables 2–4).

Discussion

Periodontal disease is a chronic infection of periodontal tissue by anaerobic Gram-negative rods, which has recently been recognised as a risk factor for PTB, IUGR, pre-eclampsia and so on.[2] *Pg* is one of the most harmful periodontal pathogens and induced IUGR in rodent models. It has been detected in the placenta of patients with pre-eclampsia and in the amniotic fluid of those with preterm labour.[4–7]

There are several studies about serum antibody levels against *Pg* during pregnancy, but the significance has been controversial. Dasanayake *et al.* stated that higher antibody

Journal compilation © 2009 The Royal Australian and New Zealand College of Obstetricians and Gynaecologists; **49:** 137–141

levels against *Pg* were associated with low birthweight deliveries.[10] On the other hand, Lin *et al.* reported that low maternal IgG antibody response to *Pg* was associated with an increased risk of PTB.[11] In our results, lower antibody response was observed in the IUGR group and some PTB patients, and these agree with Lin's data. In the previous reports described above, antibody levels were measured by the immunological method using sonicated whole bacteria as the antigen, whereas recombinant 40-kDa OMP was used in this study. 40-kDa OMP plays a role in the progression of periodontal disease caused by *Pg* and it has been suggested that 40-kDa OMP should be the antigen of a vaccination for periodontal disease.[12,13] Patients with chronic periodontitis showed significantly higher serum levels of antibody against 40-kDa OMP, especially the IgG1 subclass, than periodontally healthy controls, however, the antibody level did not have a significant association with the mean probing depth, which is one of the most reliable clinical measurements of periodontal disease.[8] Furthermore, we measured the serum level of anti-40-kDa OMP *Pg*-IgG1 of 14 non-pregnant young individuals who were periodontally healthy and *Pg* could not detected in their periodontal pockets by PCR. The median antibody level was 69.3% (IQR: 44.7~151.8%) and the highest antibody level was 195.2%, which is about twice as high as that of the control serum. Our control serum was derived from a patient with periodontitis and *Pg* was detected in her periodontal pockets by PCR. The positive rate of *Pg* by PCR in periodontal pockets was more than 50% in Japanese pregnant women in the first trimester (our unpublished data). *Pg* is the common oral bacteria in young Japanese women and most of them must have obtained humoral immunity before pregnancy. Serum CRP levels are strongly associated with periodontal status. Meta-analyses have suggested that serum CRP levels in periodontitis patients are elevated, compared with controls, and lowered by periodontal treatment.[14] In early pregnancy, compatible results were also reported.[15] Although periodontal examinations were not performed in this study, there may be no significant differences in the periodontal status between each case group and the control group because the serum CRP levels were similar in each group. These may mean that the risk factor for adverse pregnancy outcome is not periodontal disease itself but the lack of humoral immunity against *Pg* (and other oral bacteria?) in early pregnancy.

There are two hypotheses about the mechanism of periodontal disease worsening pregnancy outcomes. One explanation is intrauterine infection of oral bacteria through maternal circulation.[4–7] Maternal immunoglobulins against periodontal pathogens should protect the mother and fetus from bacterial dissemination into the uterus.[16] The other hypothesis is inflammatory cytokine or prostaglandin production in the oral cavity. Cytokines, like tumour necrosis factor-α and prostaglandin, will reach the uterus through maternal circulation and induce uterine contractions or worsen the condition of the fetus and placenta.[2,3] Boggess *et al.* reported that maternal periodontal disease in early pregnancy was associated with delivery of a small-for-gestational-age infant and hypothesised that it presents an oral microbial challenge that results in a systemic inflammatory response in a subset of women and ultimately results in abnormal placental or fetal development that impacts fetal growth.[17] In our results, it is difficult to speculate on the mechanism suppressing fetal growth, however, it is possible to do so in the case of PTB. In the PTB group, the serum levels of anti-40-kDa OMP *Pg*-IgG1 were low in patients with CAM or without *Lactobacilli* in vaginal flora. Intrauterine infection, which is called CAM pathologically, is one of the most prevalent causes of PTB and it has been suggested that the origin is often ascending infection with bacteria from the vagina and cervix.[18] *Lactobacilli* prevent pathogenic bacteria from growing in the vagina and from invading the uterus, therefore the presence of *Lactobacilli* in vaginal flora is a negative risk factor for PTB.[19] These suggested that haematogenous infection by oral bacteria may be one of the origins of intrauterine infections and occur more commonly in PTB patients who suffer from CAM despite the presence of *Lactobacilli*.

Conclusion

These results suggest that a lack of humoral immunity against *Pg* itself in early pregnancy is associated with IUGR and some PTB.

References

1 Kim J, Amar S. Periodontal disease and systemic conditions: A bidirectional relationship. *Odontology* 2006; **94**: 10–21.

2 Xiong X, Buekens P, Fraser WD, Beck J, Offenbacher S. Periodontal disease and adverse pregnancy outcomes: A systemic review. *BJOG* 2006; **113**: 135–143.

3 Shub A, Swain JP, Newnham JP. Periodontal disease and adverse pregnancy outcomes. *J Maternal Fetal Neonatal Med* 2006; **19**: 521–528.

4 Lin D, Smith MA, Elter J *et al. Porphyromonas gingivalis* infection in pregnant mice is associated with placental dissemination, an increase in the placental Th1/Th2 cytokine ratio, and fetal growth restriction. *Infect Immun* 2003; **71**: 5163–5168.

5 Lin D, Smith MA, Champagne C, Elter J, Beck J, Offenbacher S. *Porphyromonas gingivalis* infection during pregnancy increases maternal tumor necrosis factor alpha, suppresses maternal interleukin-10, and enhances fetal growth restriction and resorption in mice. *Infect Immun* 2003; **71**: 5156–5162.

6 León R, Silva N, Ovalle A *et al.* Detection of *Porphyromonas gingivalis* in the amniotic fluid in pregnant women with a diagnosis of threatened premature labor. *J Periodontol* 2007; **78**: 1249–1255.

7 Barak S, Oettinger-Barak O, Machtei EE, Sprecher H, Ohel G. Evidence of periopathogenic microorganisms in placentas of women with preeclampsia. *J Periodontol* 2007; **78**: 670–676.

8 Kobayashi T, Kaneko S, Tahara T, Hayakawa M, Abiko Y, Yoshie H. Antibody responses to *Porphyromonas gingivalis* hemagglutinin A and outer membrane protein in chronic periodontitis. *J Periodontol* 2006; **77**: 364–369.

9 Kawamoto Y, Hayakawa M, Abiko Y. Purification and immunochemical characterization of a recombinant outer membrane protein from *Bacteroides gingivalis*. *Int J Biochem* 1991; **23**: 1053–1061.

10 Dasanayake AP, Boyd D, Madianos PN, Offenbacher S, Hills E. The association between *Porphyromonas gingivalis*-specific maternal serum IgG and low birth weight. *J Periodontol* 2001; **72**: 1491–1497.
11 Lin D, Moss K, Beck JD, Hefti A, Offenbacher S. Persistently high levels of periodontal pathogens associated with preterm pregnancy outcome. *J Periodontol* 2007; **78**: 833–841.
12 Saito S, Hiratsuka K, Hayakawa M, Takiguchi H, Abiko Y. Inhibition of a *Porphyromonas gingivalis* colonizing factor between *Actinomyces viscosus* ATCC 19246 by monoclonal antibodies against recombinant 40-kDa outer-membrane protein. *Gen Pharmac* 1997; **28**: 675–680.
13 Namikoshi J, Otake S, Maeba S, Hayakawa M, Abiko Y, Yamamoto M. Specific antibodies induced by nasally administered 40-kDa outer membrane protein of Porphyromonas gingivalis inhibits coaggregation activity of *P. gingivalis*. *Vaccine* 2003; **22**: 250–256.
14 Paraskevas S, Huizinga JD, Loos BG. A systematic review and meta-analyses on C-reactive protein in relation to periodontitis. *J Clin Periodontol* 2008; **35**: 277–290.
15 Pitiphat W, Joshipura KJ, Rich-Edwards JW, Williams PL, Douglass CW, Gillman MW. Periodontitis and plasma C-reactive protein during pregnancy. *J Periodontal* 2006; **77**: 821–841.
16 Madianos PN, Lieff S, Murtha AP *et al.* Maternal periodontitis and prematurity. Part II: Maternal infection and fetal exposure. *Ann Periodontol* 2001; **6**: 175–182.
17 Bogges KA, Beck JD, Murtha AP, Moss K, Offenbacher S. Maternal periodontal disease in early pregnancy and risk for a small-for-gestational-age infant. *Am J Obstet Gynecol* 2006; **194**: 1316–1322.
18 Cunningham FG, Jeveno KJ, Bloom SL, Hauth JC, Gilstrap LC III, Wenstrom KD, eds. *Williams Obstetrics*, 22nd edn. Columbus, OH: McGraw-Hill, 2005; 176–181.
19 Hitti J, Hillier SL, Agnew KJ, Krohn MA, Reisner DP, Eschenbach DA. Vaginal indicators of amniotic fluid infection in preterm labor. *Obstet Gynecol* 2001; **97**: 211–219.

Journal compilation © 2009 The Royal Australian and New Zealand College of Obstetricians and Gynaecologists; **49:** 137–141

doi:10.1111/j.1447-0756.2008.00993.x J. Obstet. Gynaecol. Res. Vol. 35, No. 3: 593–596, June 2009

Abscess formation due to *Mycoplasma hominis* infection after cesarean section

Masayuki Yamaguchi[1], Akira Kikuchi[1], Kiyofumi Ohkusu[2], Mami Akashi[1], Jun Sasahara[1], Koichi Takakuwa[1] and Kenichi Tanaka[1]

[1]*Department of Obstetrics and Gynecology, Niigata University Graduate School of Medical and Dental Sciences, Chuo-ku, Niigata,* [2]*Department of Microbiology, Gifu University Graduate School of Medicine, Gifu, Japan*

Abstract

A 27-year-old female patient underwent cesarean section and a postoperative hematoma occurred at the site of the uterine incision. The patient underwent relaparotomy to remove the hematoma. Four days later she developed a fever of over 39°C and an abscess had formed at the site. Despite therapy with several antimicrobial agents, her fever persisted. Consequently, she underwent transvaginal abscess drainage, after which she promptly became afebrile. *Mycoplasma hominis* was considered to be the primary causative organism. There are two reasons that could explain why the wound infection became serious: (i) *M. hominis* is resistant to several antimicrobial agents that are usually used to treat obstetric infections; and (ii) a long time is required to identify the pathogen. In conclusion, *M. hominis* should be considered as a causative organism if an antimicrobial-resistant infection occurs at the surgical site after a cesarean section.

Key words: cesarean section, *Mycoplasma hominis*, postpartum infection.

Introduction

Mycoplasma hominis usually colonizes the lower urogenital tract. In the context of gynecologic and obstetric infections, there are reports of an association between *M. hominis* and pelvic inflammatory disease, postpartum fever, and preterm labor. Usually, the pathogenicity of *M. hominis* is considered to be relatively low. Although infections caused by the bacteria are often mild, occasionally, they may be serious. We report a case of abscess formation at the uterine incision site after cesarean section, which was caused by *M. hominis* infection.

Case Report

A 27-year-old woman, para 2, underwent emergency cesarean section at a local hospital because of arrest of labor at 38 weeks of gestation. A transverse incision was made at the lower uterine segment. The patient presented with hematuria 5 h after the cesarean section, and ultrasound examination revealed a 5-cm hematoma between the lower uterine segment and the bladder. Subsequently, she was transported to our hospital for further evaluation and treatment of the hematoma.

On admission, the patient's general condition was maintained, and her clinical and laboratory data were as follows: body temperature, 37°C; blood pressure, 103/63 mmHg; heart rate, 137 bpm; white blood cell count, 31 200 cells/mm^3; hemoglobin, 10.8 g/dL; and C-reactive protein (CRP), 2.3 mg/dL. The hematoma measured 8 × 6 cm and had spread into the retroperitoneal space (Fig. 1). Therefore, the patient underwent relaparotomy for hemostasis. The bleeding was identified and stemmed, and the hematoma was removed. A Penrose drain was placed at the site of the hematoma for 2 days.

Received: June 15 2008.
Accepted: August 29 2008.
Reprint request to: Dr Akira Kikuchi, Department of Obstetrics and Gynecolgy Niigata University Graduate School of Medical and Dental Sciences, 1-757 Asahimachi-dori, Chuo-ku, Niigata 951-8510, Japan. Email: no4achy@med.niigata-u.ac.jp
[Correction added after publication 18 September 2009: amendment of city name in author affiliation from Gigu to Gifu.]

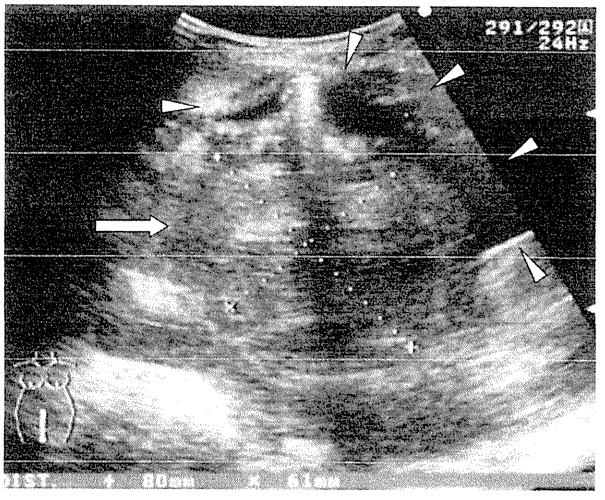


Figure 1 Ultrasonogram on admission. A hematoma was detected between the lower uterine segment and bladder (arrow). The bladder is indicated by arrowheads.

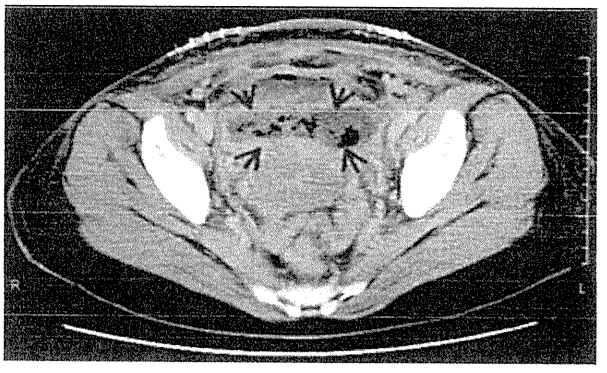


Figure 2 Computed tomography scan obtained on the fourth day. An abscess was detected at the site where the hematoma had formed (arrows).

On the second day after relaparotomy, the patient's leukocytosis and elevated CRP level showed no improvement, and on the fourth day, her body temperature rose to over 39°C. A computed tomography (CT) scan revealed a 9 × 4.5-cm abscess at the site where the hematoma had formed (Fig. 2). Although we administered cephalosporin, carbapenem, clindamycin, and aminoglycoside, the patient's high fever persisted. On the seventh day, an abdominal incisional abscess was also apparent. The removed drain and abdominal incisional abscess were plated onto blood agar and chocolate agar, and incubated at 37°C in a 5% CO_2-containing atmosphere. After 72 h of incubation, pinpoint and translucent colonies were observed on all of the plates, but repeated Gram stains of the colonies were negative. It was difficult to identify the bacterial species and perform antimicrobial sensitivity tests because the bacterial growth was very slow. However, the laboratory personnel of our department suggested that the bacteria might be *M. hominis* on the basis of the features of the colonies.

On the tenth day, the pelvic abscess was drained using the transvaginal approach. A transverse incision was made at the junction of the anterior vaginal mucosa and cervical portion of the uterus, and the bladder was dissected in order to reach the abscess wall. The abscess wall was hard, so it was punctured with a Kelly clamp under the guidance of transabdominal ultrasonography. A Penrose drain was placed in the abscess.

Fluoroquinolone (pazufloxacin mesilate) was administered, and the next day, the patient promptly became afebrile. Pazufloxacin mesilate was administered for a week, and the patient was discharged 14 days after the pelvic abscess was drained.

The Gram-negative bacteria that had been detected in the specimen obtained from the Penrose drain, the abdominal incisional abscess, and the pelvic abscess were later identified as *M. hominis* by a polymerase chain reaction (PCR) assay as described previously.[1] Briefly, bacterial cells were suspended in 300 μL of sterile water and boiled for 5 min; they were then centrifuged and the supernatant was used as the DNA template for the PCR reaction. PCR primers were used to amplify a 334-bp fragment of the *M. hominis* 16S rRNA gene as described previously.[1] A 334-bp amplicon was produced by the species-specific PCR assay. Sequencing of the amplicon demonstrated 100% base-pair identity to those of the *M. hominis* strain.

Discussion

M. hominis is a pathogenic bacterium that usually colonizes the lower urogenital tract, in which colonization is approximately 20–50%.[2–4] In the context of obstetric and gynecologic infections, *M. hominis* has been associated with pelvic inflammatory disease,[5] postpartum fever,[6] and preterm labor.[7] Furthermore, *M. hominis* causes various extragenital infections, such as septicemia,[8] septic arthritis,[9] endocarditis,[10] and brain abscess.[11]

Usually, infections caused by *M. hominis* are self-limiting and cause mild symptoms.[12–14] However, occasionally, the infection becomes serious in patients with risk factors, such as compromised immunity. In serious

cases, therapy with appropriate antibacterial agents and surgery are strongly recommended.

Mycoplasma species do not have a cell wall, which is the target for beta-lactam antimicrobials that are commonly used as prophylaxis agents for post-surgical infections. Furthermore, they do not synthesize folic acid and are resistant to antimicrobials that interfere with folic acid synthesis, such as sulfonamides.[15] Tetracyclines, erythromycin, clindamycin, chloramphenicol, aminoglycosides, and fluoroquinolones have been shown to have activity against one or more mycoplasmal species; however, there are some reports that *M. hominis* is generally resistant to erythromycins and amynoglycosides.[13,15–17] Tetracycline has been considered to be the drug of choice for treating *M. hominis* infections.[18] However, there is an increase in the emergence of tetracycline-resistant strains of *M. hominis*,[19,20] and clindamycin is often used when tetracycline is not effective.[13,15,20]

In the context of wound infection after cesarean section, Gram staining is considered a helpful method for predicting subsequent culture results.[21] However, because they lack a cell wall, mycoplasmas cannot be identified using Gram staining of clinical specimens.[16] In addition, *M. hominis* is a slow-growing bacterium, and its identification and antimicrobial sensitivity test with routine cultural methods are difficult. Therefore, appropriate antibacterial therapy is generally started late in cases of *M. hominis* infection. As noted above, a number of cases have been reported wherein *M. hominis* could not be detected by Gram staining and administration of effective antimicrobials as they were initiated late.[16,22] Furthermore, specific media were required for identification of *M. hominis* in those cases.

Recently, it was reported that compared to culture methods, PCR assay is a simple, rapid, and more sensitive method for the identification of this pathogen.[23] Therefore, a PCR assay should be performed if an *M. hominis* infection is suspected.

There are some reports on the use of surgical procedures to treat *M. hominis* infection.[13,16,20,24] In these reports, the patients underwent drainage and aspiration and received local wound care because antimicrobial therapy with beta-lactam antibiotics, such as penicillin derivatives and cephalosporins was ineffective. Surgery is considered to be a promising treatment for *M. hominis* infection if antimicrobial therapy is ineffective. In our case, antimicrobial sensitivity tests could not be performed, and it was not clear as to which antimicrobials were actually effective. Although clindamycin, which is typically considered to be effective against *M. hominis*, was administered after abscess formation, it showed no clinical effects. Once an abscess develops, antimicrobial therapies only have a limited effect even if the drug shows high *in vitro* activity; in such cases, surgery should be performed promptly.[25]

In conclusion, we present a case of severe wound infection that was caused by *M. hominis* infection after cesarean section. Resistance to beta-lactam antibiotics, which are regarded as the first-line treatment agents for antimicrobial prophylaxis, and difficulty in identification with conventional bacterial culture methods may have made the infection worse.

References

1. Blanchard A, Yáñez A, Dybvig K, Watson HL, Griffiths G, Cassell GH. Evaluation of intraspecies genetic variation within the 16S rRNA gene of *mycoplasma hominis* and detection by polymerase chain reaction. *J Clin Microbiol* 1993; **31**: 1358–1361.
2. Tully JG. Current status of the mollicute flora of humans. *Clin Infect Dis* 1993; **17** (Suppl. 1): S2–S9.
3. McCormack WM, Rosner B, Alpert S, Evrard JR, Crockett VA, Zinner SH. Vaginal colonization with *Mycoplasma hominis* and Ureaplasma urealyticum. *Sex Transm Dis* 1986; **13**: 67–70.
4. Duffy LB, Crabb D, Searcey K, Kempf MC. Comparative potency of gemifloxacin, new quinolones, macrolides, tetracycline and clindamycin against Mycoplasma spp. *J Antimicrob Chemother* 2000; **45** (Suppl. 1): 29–33.
5. Mårdh PA, Weström L. Tubal and cervical cultures in acute salpingitis with special reference to *Mycoplasma hominis* and T-strain mycoplasmas. *Br J Vener Dis* 1970; **46**: 179–186.
6. McCormack WM, Rosner B, Lee YH, Rankin JS, Lin JS. Isolation of genital mycoplasmas from blood obtained shortly after vaginal delivery. *Lancet* 1975; **1**: 596–599.
7. Usui R, Ohkuchi A, Matsubara S *et al.* Vaginal lactobacilli and preterm birth. *J Perinat Med* 2002; **30**: 458–466.
8. DeGirolami PC, Madoff S. *Mycoplasma hominis* septicemia. *J Clin Microbiol* 1982; **16**: 566–567.
9. Luttrell LM, Kanj SS, Corey GR *et al. Mycoplasma hominis* septic arthritis: Two case reports and review. *Clin Infect Dis* 1994; **19**: 1067–1070.
10. Fenollar F, Gauduchon V, Casalta JP, Lepidi H, Vandenesch F, Raoult D. Mycoplasma endocarditis: Two case reports and a review. *Clin Infect Dis* 2004; **38**: e21–e24.
11. Payan DG, Seigal N, Madoff S. Infection of a brain abscess of Mycoplasma hominis. *J Clin Microbiol* 1981; **14**: 571–573.
12. Wallace RJ Jr, Alpert S, Browne K, Lin JS, McCormack WM. Isolation of *Mycoplasma hominis* from blood cultures in patients with postpartum fever. *Obstet Gynecol* 1978; **51**: 181–185.
13. Madoff S, Hooper DC. Nongenitourinary infections caused by *Mycoplasma hominis* in adults. *Rev Infect Dis* 1988; **10**: 602–613.
14. McMahon DK, Dummer JS, Pasculle AW, Cassell G. Extragenital *Mycoplasma hominis* infections in adults. *Am J Med* 1990; **89**: 275–281.

15. McCormack WM. Susceptibility of mycoplasmas to antimicrobial agents: Clinical implications. *Clin Infect Dis* 1993; **17** (Suppl. 1): S200–S201.
16. Maccato M, Faro S, Summers KL. Wound infections after cesarean section with *Mycoplasma hominis* and *Ureaplasma urealyticum*. A report of three cases. *Diagn Microbiol Infect Dis* 1990; **13**: 363–365.
17. Mattila PS, Carlson P, Sivonen A *et al.* Life-threatening *Mycoplasma hominis* mediastinitis. *Clin Infect Dis* 1999; **29**: 1529–1537.
18. Myhre EB, Mårdh PA. Treatment of extragenital infections caused by Mycoplasma hominis. *Sex Transm Dis* 1983; **10** (4 Suppl.): 382–385.
19. Cummings MC, McCormack WM. Increase in resistance of *Mycoplasma hominis* to tetracyclines. *Antimicrob Agents Chemother* 1990; **34**: 2297–2299.
20. Zheng X, Olson DA, Tully JG *et al.* Isolation of *Mycoplasma hominis* from a brain abscess. *J Clin Microbiol* 1997; **35**: 992–994.
21. Kaplan NM, Smadi AA, Al-Taani MI *et al.* Microbiology of wound infection after cesarean section in a Jordanian hospital. *East Mediterr Health J* 2003; **9**: 1068–1074.
22. Phillips LE, Faro S, Pokorny PA *et al.* Postcesarean section wound infection by *Mycoplasma hominis* in a patient with persistent postpartum fever. *Diagn Microbiol Infect Dis* 1987; **7**: 193–197.
23. Petrikkos GL, Hadjisoteriou H, Daikos GL. PCR versus culture in the detection of vaginal *Ureaplasma urealyticum* and *Mycoplasma hominis*. *Int J Gynaecol Obstet* 2007; **97**: 202–203.
24. Payan DG, Seigal N, Madoff S. Infection of a brain abscess of Mycoplasma hominis. *J Clin Microbiol* 1981; **14**: 571–573.
25. Soper DE. Genitourinary infections and sexually transmitted diseases. In: Berek JS (ed.). *Novak's Gynecology*, 13th edn. Philadelphia, PA: Lippincott Williams & Wilkins, 2002; 453–470.

PRENATAL DIAGNOSIS
Prenat Diagn 2009; **29**: 707–709.
Published online 1 April 2009 in Wiley InterScience
(www.interscience.wiley.com) **DOI:** 10.1002/pd.2262

RESEARCH LETTER

Prenatal diagnosis of unilateral pulmonary agenesis in a pregnant woman undergoing chronic hemodialysis due to chronic renal failure

Taro Nonaka[1], Akira Kikuchi[1]*, Naoko Kido[1], Yasuhiro Takahashi[1], Kyoko Yamada[1], Tohei Usuda[2], Koichi Takakuwa[1] and Kenichi Tanaka[1]

[1]*Department of Obstetrics and Gynecology, Niigata University Graduate School of Medical and Dental Sciences, 1-757, Asahimachi-dori, Niigata, 951-8510, Japan*
[2]*Department of Pediatrics, Niigata University Graduate School of Medical and Dental Sciences, 1-757, Asahimachi-dori, Niigata, 951-8510, Japan*

KEY WORDS: pulmonary agenesis; chronic renal failure; hemodialysis; fetal ultrasound

Pulmonary agenesis is a very rare developmental malformation of the lung, in which there is a complete absence or severe hypoplasia of one or both lungs (Bianchi *et al.*, 2000). Prognosis of pulmonary agenesis varies from stillbirth, to neonatal death, to survival without any symptoms (Booth *et al.*, 1967; Maltz *et al.*, 1968; Engellenner *et al.*, 1989). We describe the prenatal diagnosis of unilateral pulmonary agenesis in a woman with chronic renal failure (CRF) treated with hemodialysis.

A 32-year-old woman, para 0, had undergone hemodialysis for 6 months because of CRF, the cause of which was unknown. She conceived spontaneously and was referred to our hospital at 22 weeks' gestation for the management of pregnancy and delivery. She had no family history of any malformation. At sonographic scan, the fetal cardiothoracic area ratio (CTAR) was increased to 38%. No structural cardiac anomalies were observed. The total cardiac dimension was normal (19 mm) and the thoracic circumference/abdominal circumference ratio was low (0.65), which suggested not a cardiomegaly but a small thorax. A mediastinal shift to the right side was present without abnormal intrathoracic mass lesions. The left lung appeared normal, however, the right lung could not be detected (Figure 1a). Therefore, we diagnosed the fetus as having right pulmonary agenesis. Magnetic resonance imaging (MRI) examinations at 26 and 35 weeks' gestation confirmed the absence of the right fetal lung without abnormal intrathoracic masses (Figure 1a and b). Her pregnancy course was uneventful, and preterm labor and polyhydroamnios were not observed. She underwent hemodialysis four times a week and after 24 weeks' gestation, hemodialysis was performed five times a week. Maternal renal function was stable during the pregnant period. Her fetus was healthy and fetal growth was appropriate. At 35 weeks' gestation, MRI was performed again for reevaluation of fetal lungs, and the lack of the right lung was confirmed, as suspected from the MRI examination performed at 26 weeks' gestation (Figure 1b). At 40 weeks' gestation, she delivered a male infant (2834 g) vaginally. The apgar score at 1 min was 4 points, so mask ventilation was performed and the apgar score improved to 8 points at 5 min. A plain chest radiogram showed hyperinflation of the left lung, a mediastinal shift toward the right side, and radiopacity of the right hemithorax. Administration of oxygen and nasal directional positive airway pressure were required for a few days due to cyanosis, retraction, and low oxygen saturation of pulse oximetry, after which no treatment was necessary. Computed tomography (CT) was performed on the fourth day after birth and the results again confirmed the infant's right pulmonary agenesis. No associated anomalies were detected by physical exam, CT, or ultrasound examination of the brain, heart, and abdomen. Mother and child were discharged from our hospital, 14 days after delivery. At pediatric follow-up after 1 year, the child was doing well.

Pulmonary agenesis is defined as the complete absence or severe hypoplasia of one or both lungs (Bianchi *et al.*, 2000). Pulmonary agenesis is considered to be a rare malformation. Schechter *et al.* (1968) estimated an incidence of 1 in 15 000 based on autopsies. There have been some reports on prenatal diagnosis of unilateral pulmonary agenesis (Bianchi *et al.*, 2000; Viora *et al.*, 2002). There has been only one reported case that was diagnosed prenatally during the second trimester at 23 weeks' gestation (Viora *et al.*, 2002). The unilateral pulmonary agenesis of our case was prenatally diagnosed at 22 weeks and may be the earliest such case in the literature. We suspect that prenatal diagnosis of unilateral lung agenesis should be possible even before 22 weeks' gestation. The most important sonographic finding is usually the mediastinal shift to the affected

*Correspondence to: Akira Kikuchi, Department of Obstetrics and Gynecology, Niigata University Graduate School of Medical and Dental Sciences, 1-757, Asahimachi-dori, Niigata City, Niigata 951-8510, Japan. E-mail: no4achy@med.niigata-u.ac.jp

Received: 14 October 2008
Revised: 13 February 2009
Accepted: 14 February 2009
Published online: 1 April 2009

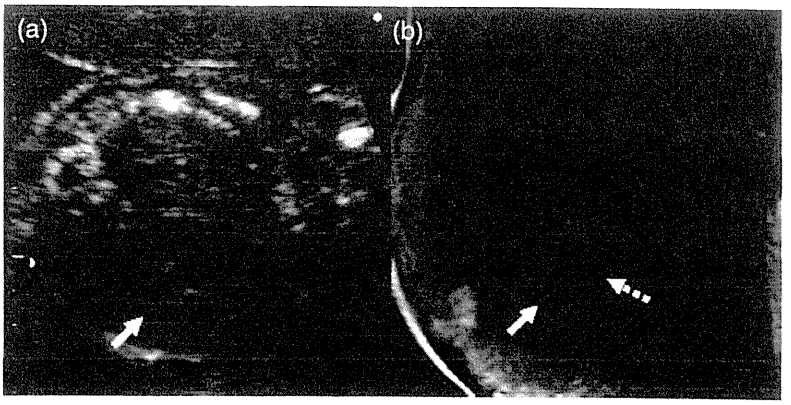


Figure 1—(a) Coronal section of fetal thorax (4-chamber view) at 22 weeks' gestation by ultrasound. The right lung could not be detected and the heart (solid arrow) was shifted to the right side. The cardiothoracic area ratio was increased to 38%. (b) MRI (half Fourier single-shot Turbo spin echo) performed at 35 weeks' gestation. Right lung was absent and the mediastinum (solid arrow) was shifted toward the right side. Dotted arrow indicates left lung

side. Therefore, differential diagnoses have been considered, that is, an abnormal intrathoracic mass lesion, such as a congenital diaphragmatic hernia or a congenital cystic adenomatoid malformation of the lung (Bianchi *et al.*, 2000). In this case, at first we noticed an increase in CTAR, but not a mediastinal shift. If increased CTAR is observed, which was induced by the lack of a unilateral lung in this case, the fetal condition causing increased CTAR, such as a congenital cardiac disease, should be considered as a differential diagnosis. MRI is more useful than ultrasound in identifying fetal thoracic organs. Indeed, MRI, which clearly demonstrated the absence of the right lung and abnormal mass lesions, was helpful in confirming the diagnosis of pulmonary agenesis.

Bilateral agenesis of the lung is incompatible with life. Unlike bilateral lung agenesis, unilateral pulmonary agenesis is not lethal. Some patients may die in the neonatal period because of respiratory failure or pulmonary hypoplasia, others have only modest and transient respiratory distress at birth like our case, or signs of illness may be absent in the newborn. However, even these infants remain at risk for recurrent bronchopulmonary infection, which can be a cause of death (Bianchi *et al.*, 2000; Hansen *et al.*, 2005). About half of the cases have been associated with other malformations, including those of the cardiovascular, gastrointestinal, genitourinary, ocular, and skeletal systems. Another common cause of death is related to associated malformations, primarily cardiac in cause. Without major malformations and problems of the respiratory system, long-term survival is possible (Berkenstadt *et al.*, 1999; Bianchi *et al.*, 2000).

In our case, fetal pulmonary agenesis occurred in a pregnant woman undergoing hemodialysis due to CRF. CRF requiring hemodialysis can be associated with fetal growth restriction (Chao *et al.*, 2002) and with high risk of fetal congenital anomalies (Okundaye *et al.*, 1998). A careful ultrasound examination for evaluating fetal growth and malformations, including pulmonary agenesis, is recommended for pregnant women undergoing hemodialysis.

Unilateral pulmonary agenesis occurs approximately 25 times more commonly than bilateral cases (Booth *et al.*, 1967; Maltz *et al.*, 1968). It is more difficult to diagnose prenatally the absence of one lung compared to that of both lungs by ultrasound. Unilateral pulmonary agenesis presents the possibility of several associated diseases, which need to be distinguished. Neonates with unilateral pulmonary agenesis often have some problems in regard to respiration and associated anomalies (Bianchi *et al.*, 2000). In unilateral pulmonary agenesis, prenatal evaluation of the associated anomalies and early treatment immediately after birth by a neonatologist can improve the prognosis. If prenatal diagnosis is made, management of pregnancy and delivery should be done in a tertiary care center.

In conclusion, we reported here a case of unilateral pulmonary agenesis in a pregnant woman receiving hemodialysis. Prenatal diagnosis is important to improve the prognosis of unilateral pulmonary agenesis.

REFERENCES

Berkenstadt M, Lev D, Achiron R, Ronser M, Barkai G. 1999. Pulmonary agenesis, microphthalmia and diaphragmatic defect (PMD): new syndrome or association? *Am J Med Genet* **86**: 6–8.

Bianchi DW, Crombleholme TM, D'Alton ME. 2000. Pulmonary agenesis. In *Fetology Diagnosis and Management of the Fetal Patient*, Bianchi DW, Chrombelholme TM, D'Alton ME (eds). McGraw-Hill: New York; 323–328.

Booth JB, Berry CL. 1967. Unilateral pulmonary agenesis. *Arch Dis Child* **42**: 361–363.

Chao AS, Huang JY, Lien R, Kung FT, Chen PJ, Hsieh PC. 2002. Pregnancy in women who undergo long-term hemodialysis. *Am J Obstet Gynecol* **187**(1): 152–156.

Engellenner W, Kaplan C, Van de Vegte GL. 1989. Pulmonary agenesis association with non-immune hydrops. *Pediatr Pathol* **9**: 725–730.

Hansen TN, Corbet A. 2005. Anomalies of the Airways, Mediastinum, and Lung Parenchyma. In *Avery's Diseases of the Newborn*, Taeusch HW, Ballard RA, Gleason GA (eds). ELSEVIER SAUNDERS: Philadelphia, PA; 737–757.

Maltz DL, Nadas AS. 1968. Agenesis of the lung: presentation of eight new cases and review of the literature. *Pediatrics* **42**: 175–185.

Prenat Diagn 2009; **29**: 707–709.
DOI: 10.1002/pd