Nocturnal intermittent hypoxia as an associated risk factor for microalbuminuria in Japanese patients with type 2 diabetes mellitus

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Abstract

Objective: We estimated the prevalence of nocturnal intermittent hypoxia, a surrogate marker of obstructive sleep apnoea, among type 2 diabetes mellitus (T2DM) patients and examined the association between nocturnal intermittent hypoxia and microvascular diseases.

Design and methods: We recruited 513 Japanese patients (292 men and 221 women) with T2DM. Nocturnal intermittent hypoxia was diagnosed using the 3% oxygen desaturation index, with less than five events per hour corresponding to normal and five events or more per hour corresponding to nocturnal intermittent hypoxia.

Results: The prevalence of nocturnal intermittent hypoxia was 45.4% among T2DM patients. The nocturnal intermittent hypoxia group was older and had a higher BMI, greater weight change since the age of 20 years, higher smoking rate and increased prevalence of hypertension, hyperlipidaemia, microalbuminuria and macroalbuminuria. Microalbuminuria (model 1: odds ratio (OR), 3.41; 95% CI, 1.85–6.40; model 2: OR, 3.69; 95% CI, 1.85–7.59 and model 3: OR, 3.12; 95% CI, 1.45–6.95) and nephropathy (model 1: OR, 4.51; 95% CI, 1.58–15.1; model 2: OR, 7.31; 95% CI, 2.11–31.6 and model 3: OR, 5.23; 95% CI, 1.45–23.8) were derived as factors from all the three statistical models and constantly associated with nocturnal intermittent hypoxia only in women.

Conclusions: Nocturnal intermittent hypoxia was highly prevalent among T2DM patients and may be an independent associated risk factor for microalbuminuria in Japanese women with T2DM.

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Introduction

The prevalence of type 2 diabetes mellitus (T2DM) worldwide was 2.8% in 2000 and is estimated to increase to 4.4% by 2030 owing to an overwhelming increase in the obese population (1). The increase in the prevalence of T2DM is a serious burden to public health because this disease is not only a risk factor for macro- and microvascular disorders but also associated with all-cause mortality (2). The current obesity epidemic has contributed to an increase in the prevalence of T2DM in Japan (3). T2DM is the leading cause of haemodialysis and blindness in Japan, and recent cohort studies have confirmed that T2DM is associated with an increased risk of coronary heart disease and all types of stroke, except haemorrhagic stroke (4).

The International Diabetes Federation has expressed the need for further research into the links between sleep-disordered breathing (SDB) and T2DM (5). Several cross-sectional studies have indicated that increases in the apnoea–hypopnoea index are independently associated with insulin resistance. In the Wisconsin Sleep Cohort Study conducted in a prospective longitudinal manner, the prevalence of DM was three to four times more in subjects with severe SDB than in those without SDB (6). However, SDB was not a causal factor in the development of T2DM after adjustment for age, sex and waist circumference (6). Meanwhile, nocturnal intermittent hypoxia, which is a surrogate marker of obstructive sleep apnoea (OSA), was reported to be associated with an increased risk for developing T2DM among middle-aged Japanese patients (7).
Therefore, the role of SDB in the development of diabetes may differ among racial groups.

Studies on the association between SDB and microvascular complications among T2DM patients are limited, and only a few studies have investigated SDB in Japanese diabetic subjects. A relationship between severe SDB and diabetic retinopathy among 166 Japanese diabetic subjects was identified in a cross-sectional case–control study (8). Meanwhile, Kashine et al. (9) investigated SDB and clinical factors in 40 Japanese diabetic subjects and found no association between SDB and complications of T2DM. The sample size of these previous studies was insufficient to identify relationships between SDB and complications of T2DM.

SDB is very common among patients with chronic kidney diseases (10, 11). Nocturnal hypoxia is associated with an increased risk of kidney function loss (12, 13), and short-duration continuous positive airway pressure (CPAP) therapy can be used for the treatment of microalbuminuria (14). However, the influence of nocturnal intermittent hypoxia on microalbuminuria among T2DM patients remains unknown.

In this study, we hypothesised that nocturnal intermittent hypoxia, as a surrogate marker of OSA, may be a potential and associated risk factor for microvascular complications. The aims of this study were to estimate the prevalence of nocturnal intermittent hypoxia among patients with T2DM and to examine the association between nocturnal intermittent hypoxia and microvascular diseases.

Subjects and methods

Study population

The Dogo Study is a multicentre prospective cohort study that recruited 513 Japanese patients with previously diagnosed T2DM (median age at recruitment, 62.0 years; range, 20–85 years; men, 292 and women, 221) whose sleep pattern was assessed between September 2009 and December 2010. The Dogo Study Group consists of medical doctors who specialise in DM at ten hospitals in Ehime prefecture, Japan. T2DM was diagnosed according to the Japan Diabetes Society criteria (15). This study protocol received ethical approval from the institutional review board of Ehime University, functioning according to the 3rd edition of the Guidelines on the Practice of Ethical Committees in Medical Research issued by the Royal College of Physicians of London. Written informed consent was obtained from all patients after full explanation of the purpose and nature of all procedures used.

Measurements

All participants completed self-administered questionnaires about weight at the age of 20 years, the duration of diabetes, alcohol intake per day, numbers of cigarettes smoked per day, use of antihypertensive medication, use of antihyperlipidaemic medication and the Epworth sleepiness scale (ESS, eight items with a value of 0–3 for each and a total score of 0–24) (16). BMI was calculated as weight (kg) divided by square of height in metres (m²). Weight change since the age of 20 years was estimated using the current body weight and self-reported body weight at the age of 20 years.

Patients who smoked ≥1 cigarette/day were regarded as current smokers. ESS scores ≥11 were considered to represent excessive daytime sleepiness, as shown by previous studies (16, 17). Blood pressure was measured with a cuff in the sitting position after a rest period of more than 5 min. Hypertension was defined as a systolic blood pressure of more than 140 mmHg or a diastolic blood pressure of more than 90 mmHg, or both, or if the patients were already receiving antihypertensive drugs. Hyperlipidaemia was defined as a serum total cholesterol concentration of >5.69 mmol/l, a triglyceride concentration of >1.69 mmol/l or a HDL-cholesterol concentration of <1.03 mmol/l, or if the patients were already being treated with lipid-lowering agents. Stroke and ischaemic heart disease were assessed using the results of self-administered questionnaires, medical records and admission data.

Assessments of complications of T2DM

The complications of microvascular disease in T2DM are retinopathy, nephropathy and neuropathy; these complications were assessed using the following definitions. The diagnosis of retinopathy was indicated by the presence of haemorrhages, microaneurysms, soft and hard exudates, areas of neovascularisation or laser coagulation scars in at least one eye using florescence fundoscopy on dilated pupils within 3 months of recruitment. Several ophthalmology specialists checked the fundus of all participants. All ophthalmologists were blinded to the diagnosis of nocturnal intermittent hypoxia. Nephropathy was defined using the urinary albumin:creatinine ratio (UACR) to classify the participants as follows: normoalbuminuria, <3.4 mg/mmol creatinine; microalbuminuria, ≥3.4 mg/mmol creatinine and nephropathy, ≥34 mg/mmol creatinine. UACR was calculated using the urine sample taken on the first morning (18). Diabetic neuropathy was defined if the patients showed two or more of the following three characteristics: neuropathic symptoms, the absence of ankle tendon reflexes and abnormal vibration perception threshold scores assessed with a 128 Hz tuning fork (19).

Assessments of nocturnal intermittent hypoxia

A pulse oximeter (PULSOX-3Si; Minolta Co., Osaka, Japan) was attached to the left wrist during one night
of sleep. The sensor probe was fitted to the ring finger and secured with a tape by each subject. The internal memory of this device stores the values of blood oxygen saturation by performing a moving average for the last 5 s, updated every second; the sampling time was short in order to avoid underestimation of oxygen desaturation (20). Data were downloaded to a personal computer via an interface (PULSOX IF-3; Minolta Co.) and were analysed using proprietary software supplied with the equipment (DS-3 version. 2.0a; Minolta Co.). We used the 3% oxygen desaturation index (ODI) as an indicator of nocturnal intermittent hypoxia. The value of 3% ODI was taken as the mean value over a period of sleep of at least 4 h, as estimated by pulse oximetry. The severity of nocturnal intermittent hypoxia was defined by the 3% ODI level: normal, less than five events per hour; and nocturnal intermittent hypoxia, five events or more per hour (21, 22).

**Statistical analysis**

All numerical variables are expressed as mean ± s.d. Statistical analysis was conducted using one-way ANOVA. We used multivariate logistic regression models to estimate the odds ratio (OR) for the presence of microalbuminuria, nephropathy, retinopathy and neuropathy. All statistical analyses were performed using JMP 9 (SAS Institute, Inc., Cary, NC, USA). All probability values for statistical tests were two tailed, and P values of < 0.05 were regarded as statistically significant.

**Results**

As shown in Table 1, the prevalence of nocturnal intermittent hypoxia was 45.4% among patients with T2DM. The group of patients with nocturnal intermittent hypoxia was older and had a higher BMI, a greater weight change since the age of 20 years, a higher smoking rate and an increased prevalence of hypertension, hyperlipidaemia, microalbuminuria and macroalbuminuria. Similarly, significant differences were observed between men in the nocturnal intermittent hypoxia group and the normal group in terms of BMI, weight change since the age of 20 years, smoking rate, hypertension, microalbuminuria and macroalbuminuria. Meanwhile, significant differences were also found between women in the nocturnal intermittent hypoxia group and the normal group in regard to age, BMI, weight change since the age of 20 years, creatinine, hypertension, hyperlipidaemia, microalbuminuria and macroalbuminuria.

Table 2 shows the relationship between nocturnal intermittent hypoxia and microvascular complications. In the sex- and age-adjusted model for ODI ≥ 5 events/h (model 1), only the ORs for nephropathy (OR, 3.25; 95% CI, 1.67–6.69) and microalbuminuria (OR, 2.18; 95% CI, 1.49–3.20) showed a positive association, whereas retinopathy and neuropathy did not. After adjustment for sex, age, BMI, hypertension and hyperlipidaemia for ODI ≥ 5 events/h (model 2), the ORs for nephropathy (OR, 2.84; 95% CI, 1.38–6.18) and microalbuminuria (OR, 1.76; 95% CI, 1.16–2.69) were significant. After adjustment for sex, age, BMI, hypertension, hyperlipidaemia, current smoking, current drinking, stroke, ischaemic heart disease, duration of T2DM and HbA1c (model 3), the multivariate analysis identified ODI ≥ 5 events/h to be associated with microalbuminuria (OR, 1.84; 95% CI, 1.16–2.96) and nephropathy (OR, 2.97; 95% CI, 1.36–6.90). Microalbuminuria (OR, 1.64; 95% CI, 1.11–2.68) and nephropathy (OR, 2.62; 95% CI, 1.12–6.68) derived from model 1 showed an association with ODI ≥ 5 events/h in men, but these associations were negated in models 2 and 3. By contrast, microalbuminuria (model 1: OR, 3.41; 95% CI, 1.86–6.40; model 2: OR, 3.69; 95% CI, 1.85–7.59 and model 3: OR, 3.12; 95% CI, 1.45–6.95) and nephropathy (model 1: OR, 4.51; 95% CI, 1.58–15.1; model 2: OR, 7.31; 95% CI, 2.11–31.6 and model 3: OR, 5.23; 95% CI, 1.45–23.8) were derived as factors from all three models and constantly associated with nocturnal intermittent hypoxia in women. However, retinopathy and neuropathy did not show any associations with nocturnal intermittent hypoxia in both sexes.

Table 3 shows the diabetic nephropathy ODI ≥ 5 events/h ORs according to the duration of T2DM and the weight change since the age of 20 years. The ODI ≥ 5 events/h OR for microalbuminuria was 1.94 (95% CI, 1.12–3.39) for those patients with diabetes for a duration < 11 years and 2.69 (95% CI, 1.57–4.68) for those with diabetes ≥ 11 years. Similarly, the ODI ≥ 5 events/h OR for nephropathy was 2.69 (95% CI, 1.76–11.1) among those with a duration of ≥ 11 years. The OR for the association between weight change since the age of 20 years and microalbuminuria were significantly high in the following categories: weight change ≥ 0 kg (OR, 2.57; 95% CI, 1.29–5.29) and ≥ 10 kg (OR, 2.27; 95% CI, 1.17–4.72), while the association between weight change since the age of 20 years and diabetic nephropathy were only observed in the category of weight change ≥ 0 kg (OR, 7.79; 95% CI, 1.87–53.3).

Table 3 shows ODI ≥ 5 events/h OR for microalbuminuria and diabetic nephropathy among patients with T2DM in men. No differences were observed between the categories for the duration of T2DM and weight change since the age of 20 years. Table 3 shows ODI ≥ 5 events/h OR for microalbuminuria and diabetic nephropathy among patients with T2DM in women. The ODI ≥ 5 events/h OR for microalbuminuria were 2.33 (95% CI, 1.06–5.24) and 6.02 (95% CI, 2.26–17.9) among patients with T2DM of a duration of < 11 years and ≥ 11 years respectively. Similarly, the ODI ≥ 5 events/h OR for nephropathy was 3.76 (95% CI, 2.21–31.0) among those with a duration of ≥ 11 years. The OR for the association between weight...
Table 1 Clinical characteristics of the total patient population and patients according to gender.

<table>
<thead>
<tr>
<th></th>
<th>Total (3% ODI, events/h)</th>
<th>Men (3% ODI, events/h)</th>
<th>Female (3% ODI, events/h)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(n=513) 0–4.9 (n=280) ≥ 5 (n=233)</td>
<td>(n=292) 0–4.9 (n=159) ≥ 5 (n=133)</td>
<td>(n=221) 0–4.9 (n=121) ≥ 5 (n=100)</td>
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<tr>
<td>Demographics</td>
<td></td>
<td></td>
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<tr>
<td>Age (years)</td>
<td>62.0 ± 10.5</td>
<td>60.9 ± 10.8</td>
<td>63.4 ± 10.0*</td>
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<tr>
<td>Gender: male (%)</td>
<td>56.9</td>
<td>56.7</td>
<td>57.3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.2 ± 4.9</td>
<td>23.8 ± 4.2</td>
<td>26.7 ± 5.3*</td>
</tr>
<tr>
<td>BMI at the age of 20 years (kg/m²)</td>
<td>21.9 ± 4.1</td>
<td>22.0 ± 3.7</td>
<td>21.9 ± 4.7</td>
</tr>
<tr>
<td>Weight change since the age of 20 years (kg)</td>
<td>7.8 ± 14.0</td>
<td>4.4 ± 13.0</td>
<td>11.4 ± 14.5*</td>
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<td>Creatinine (μmol/l)</td>
<td>71.59 ± 32.83</td>
<td>67.43 ± 19.47</td>
<td>71.78 ± 22.15</td>
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<td>HbA1c (%)</td>
<td>7.29 ± 1.54</td>
<td>7.26 ± 1.52</td>
<td>7.38 ± 1.63</td>
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<tr>
<td>Duration of T2DM (years)</td>
<td>11.7 ± 9.5</td>
<td>11.3 ± 9.4</td>
<td>12.3 ± 9.5</td>
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<tr>
<td>Current smoker (%)</td>
<td>21.1</td>
<td>11.5</td>
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<td>Regular alcohol drinker (%)</td>
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<td>Epworth sleepiness scale</td>
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<td>36.8</td>
<td>58.8*</td>
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<td>Hypertension (%)</td>
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<td>35.6</td>
<td>54.9*</td>
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<tr>
<td>Hyperlipidaemia (%)</td>
<td>5.1</td>
<td>3.6</td>
<td>6.9</td>
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<tr>
<td>Stroke (%)</td>
<td>8.8</td>
<td>7.2</td>
<td>10.4</td>
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<tr>
<td>Ischaemic heart disease (%)</td>
<td></td>
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<tr>
<td>Complications of T2DM</td>
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<td></td>
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<tr>
<td>Microalbuminuria (%)</td>
<td>23.6</td>
<td>17.5</td>
<td>31.2*</td>
</tr>
<tr>
<td>Nephropathy (macroalbuminuria) (%)</td>
<td>8.6</td>
<td>4.8</td>
<td>13.0*</td>
</tr>
<tr>
<td>Retinopathy (%)</td>
<td>23.1</td>
<td>21.8</td>
<td>24.2</td>
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<tr>
<td>Neuropathy (%)</td>
<td>47.5</td>
<td>44.6</td>
<td>51.2</td>
</tr>
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</table>

T2DM, type 2 diabetes mellitus; ODI, oxygen desaturation index. *P<0.05, nocturnal intermittent hypoxia vs normal in each category.
change since the age of 20 years and microalbuminuria were significant in the following categories: weight change ≥0 kg (OR, 6.00; 95% CI, 1.82–21.9) and ≥10 kg (OR, 3.68; 95% CI, 1.52–9.70). Similarly, the OR for the association between weight change since the age of 20 years and nephropathy were significant in the following categories: weight change ≥0 kg (OR, 6.20; 95% CI, 1.12–114.2) and ≥10 kg (OR, 6.99; 95% CI, 1.21–133.0).

### Discussion

Our results show that nocturnal intermittent hypoxia, defined as 3% ODI ≥5 events/h, was associated with the presence of diabetic nephropathy among 513 patients with T2DM. The prevalence of nocturnal intermittent hypoxia among patients with T2DM was 45.5% in this study (45.5% in men and 45.2% in women). Although we did not have a control group to assess the precise prevalence of nocturnal intermittent hypoxia among patients with T2DM, this group of subjects is likely to have higher nocturnal intermittent hypoxia than the general population.

An association between insulin resistance and microalbuminuria in T2DM has often been found in cross-sectional studies. In Taiwan, insulin resistance was reported as a predictive marker for the development of microalbuminuria in patients with T2DM (23). SDB is thought to cause insulin resistance via the following three steps. First, reduced oxygen desaturation stimulates sympathetic nerves. This stimulation is mediated by peripheral arterial chemoreceptors, particularly carotid bodies, with enhanced sympathetic drive apparently persisting during normal waking hours (24). Enhanced sympathetic drive raises serum catecholamine levels, leading to elevated serum glucose levels (25). Secondly, elevated oxidative stress raises cytokine levels (26), leading to increased insulin resistance (27). Thirdly, sleep fragmentation has been found to activate the hypothalamic–pituitary–adrenal axis (28), resulting in a marked elevation in serum cortisol levels, thereby leading to insulin resistance (29).

Furthermore, chronic hypoxia has been identified as a key regulator in diabetic nephropathy (30), which is associated with the activation of hypoxia-inducible factor 1α (HIF1α). HIF1α plays an important role in diabetic nephropathy (31, 32, 33), showing higher expression in diabetic kidneys than in normal human kidneys (34), and it has been reported as a novel target for the remission of diabetic nephropathy (35).

Of interest, cell culture experiments have shown the upregulation of HIF1α in intermittent hypoxia, while elevated serum levels of HIF1α gene products, such as EPO and vascular endothelial growth factor, have been demonstrated in OSA syndrome patients, particularly those with severe nocturnal hypoaxaemia (36, 37, 38, 39).

According to our results, the relationship between other complications of T2DM and nocturnal intermittent hypoxia was not significant. Previous studies showed that lower BMI was a risk factor for retinopathy (40, 41), while nocturnal intermittent hypoxia showed a strong relationship with obesity. Therefore, the influence of nocturnal intermittent hypoxia on diabetic retinopathy may be weak. However, the mechanism underlying the association between lower BMI and diabetic retinopathy is unclear. The mean duration of T2DM in our population was more than 11 years, and therefore, the prevalence of diabetic neuropathy in our study was high. The association between nocturnal intermittent hypoxia and neuropathy may not be significant.
Table 3 shows that nocturnal intermittent hypoxia leads to microalbuminuria and diabetic nephropathy in patients with long-standing T2DM. Furthermore, the impact of nocturnal intermittent hypoxia was worse in obese patients. Moreover, this finding was rather significant in female patients. Long-term weight gain from adulthood to middle age increases the risk of T2DM in Japanese men and women. However, the risk of T2DM is further enhanced by weight gain in later life only in women (42). This may explain why nocturnal intermittent hypoxia affects microalbuminuria only in women. Meanwhile, hypertension may be a stronger risk factor for microalbuminuria in men as reported previously (43). To facilitate the remission and regression of microalbuminuria, it is important to perform CPAP therapy in the early stage of diabetic nephropathy and maintain adequate levels of plasma glucose and a healthy weight.

We acknowledge that our research had several limitations in terms of the study design. First and most importantly, our study did not have a control group to compare the prevalence of nocturnal intermittent hypoxia. However, the prevalence of nocturnal intermittent hypoxia in community-based Japanese men and women is reported to be 37.7–45.3% and 17.7–19.5% respectively (7). These results indicate that the prevalence of nocturnal intermittent hypoxia among T2DM may be higher than that in non-diabetic individuals. Secondly, our study was cross-sectional, and we used a portable screening device to assess nocturnal intermittent hypoxia. Polysomnography is the gold standard for diagnosing OSA and is useful for evaluating nocturnal intermittent hypoxia (44). However, polysomnography is expensive, time-consuming and unsuitable for screening. Hence, we used reliable pulse oximetry to estimate nocturnal intermittent hypoxia (21). The validity of the pulse oximetry was confirmed by synchronous overnight recording of both PULSOX-3Si and standard polysomnography among 256 consecutive patients who had been followed at an SDB centre in Japan (21). However, we were able to measure ODI only on one night at the patient’s home and could not evaluate the ODI during the same day of the week. Finally, we obtained data on the weight change from 20 years of age from questionnaires.

Further, we were unable to perform renal biopsies for the diagnosis of diabetic nephropathy in all patients for ethical reasons. Finally, most of the participants had already been treated for diabetes for several years. Nevertheless, our data suggest that T2DM female patients with microalbuminuria undergo SDB screening and that nocturnal intermittent hypoxia should be regarded as a target to prevent end-stage renal disease, regardless of symptoms.

In conclusion, nocturnal intermittent hypoxia may be an independent associated risk factor for...
microalbuminuria in Japanese women with T2DM. Our study is the first report to show the relationship between nocturnal intermittent hypoxia and renal function among T2DM patients. Further longitudinal research is necessary to determine the relationship between nocturnal intermittent hypoxia and diabetic nephropathy and to elucidate the effects of CPAP on diabetic nephropathy.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement
S Furukawa, S Yamamoto, T Miyake, T Niiya, T Ueda, M Torisu, T Sakai, H Minami, H Miyaoka, S Sakurai and M Matsuura conducted the research; S Furukawa, T Kugimi, S Saadao and T Tanigawa reviewed and edited the manuscript and M Onji reviewed the manuscript.

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