Title: A urine biomarker for severe OSA patients: Lipocaline-type prostaglandin D synthase

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Supplementary material

METHODS

Study subjects

Exclusion criteria

Exclusion criteria were history of CVD; acute infection; malignancy; inflammatory, autoimmune, or other chronic diseases; diabetes mellitus (DM) under treatment with hypoglycemic agents or insulin; use of steroids, non-steroidal anti-inflammatory drugs, or immunosuppressive drugs; renal dysfunction (serum creatinine ≥ 1.2 mg/dl); respiratory diseases such as bronchial asthma or chronic obstructive pulmonary disease; endocrine disorders; neurological disorders; and being a current smoker. In addition, patients were excluded if they had predominantly central sleep apnoea.

PSG

The diagnosis of OSA was confirmed by PSG (SomnoStar pro, Cardinal Health, Dublin, OH, USA), which was started at 22:00 and ended at 6:00 the following morning. Surface electrodes were attached using standard techniques to obtain an electrooculogram, electromyogram of the chin, and 12-lead electroencephalograph. Sleep stages were defined according to the criteria of Rechtschaffen and Kales [S1]. Ventilation was monitored by

inductive plethysmography (Respitrace QDC, Viasys Healthcare, Palm Springs, CA, USA). Airflow was monitored by a nasal pressure transducer (PTAFlite, Pro-Tech Services, Inc., Mukilteo, WA, USA) and supplemented by an oronasal thermal sensor (Sleepmate Technologies, Midlothian, VA, USA). Arterial oxygen saturation (SaO_2) was monitored continuously with a pulse oximeter (Adult Flex System, Nonin Medical, Plymouth, MN, USA).

Apnoea was defined as the complete cessation of airflow and hypopnoea as a clear decrease in airflow of 50% or more lasting for 10 seconds or more accompanied by a decrease in percutaneous oxygen saturation (SpO_2) of at least 3% and/or associated with arousal [S2]. All apnoea-hypopnoea index (AHI) values were expressed as the number of episodes of apnoea and hypopnoea per hour over the total sleep time. The lowest SpO_2 during sleep was calculated in each patient. Arousals were scored using the American Sleep Disorders Association's 3-second definition [S3], and the arousal index was calculated according to the number of arousals per hour of sleep. Nocturnal oxygen desaturation was assessed by the minimum SpO_2 during sleep and $SpO_2 < 90\%$ time per total sleep time (TST) ($SpO_2 < 90\%$, %TST). OSA severity was defined by the AHI as follows: control ($AHI < 15$), moderate OSA ($15 \leq AHI < 30$), and severe OSA ($AHI \geq 30$).

Measurements of plasma and urinary L-PGDS concentrations

Concentrations of urinary or plasma L-PGDS were measured by an enzyme-linked immunosorbent assay (ELISA) using 2 monoclonal antibodies, Mab-7F5 and Mab-1B7, as described previously [S4-S7]

In brief, urine or serum (plasma) samples were diluted [urine: 1:100-800 (1 part urine sample plus 99-799 parts diluent), serum (plasma): 1:50-400 (1 part serum or plasma sample plus 49-399 parts diluent)] with Tris-buffered saline (20 mM Tris-HCl, pH 7.4, and 154 mM NaCl) containing 1 g/L bovine serum albumin and 0.1 g/L thimerosal and incubated at 25°C for 90 min in Costar 3590 microtiter plates (Corning, Corning, NY, USA) precoated with unlabelled Mab-7F5 (10 mg/L). After a wash, the plates were incubated at 25°C with peroxidase-conjugated Mab-1B7 for 90 min. Thereafter, the substrate solution containing 3,3', 5, 5'-tetramethylbenzidine (BM blue-POD substrate; Roche Diagnostics, Mannheim, Germany) was added to each well, and the reaction was stopped by adding 1 mol/L sulfuric acid (100 μL/well).

The plates were read using a Spectra-Max 250 microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 450 nm. Recoveries of L-PGDS in urine samples and serum samples ranged from 91% to 111% and from 91% to 109%, respectively [S6, S7]. When serum samples containing 0.43, 0.98, and 1.15 mg/L were serially diluted and assayed, each sample gave results close to linearity (r =0.999-1.000) [S7]. When urine samples containing 0.9, 2.7, and 4.7 mg/L L-PGDS were serially diluted and assayed, each sample gave results close to linearity (r

=0.998–1.000), confirming parallelism between the calibrators and urine samples [S6]. With this ELISA system, it has been demonstrated that intra- and interassay coefficients of variation in urine samples ranged from 3.2% to 5.8% and from 7.6% to 8.3%, respectively [S6]. The intra- and interassay coefficients of variation in serum samples were 3.6% and 5.8%, respectively [S7]. All of the samples were measured in duplicate and the results were averaged.

References (Supplementary material)

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S4 Eguchi Y, Eguchi N, Oda H, Seiki K, Kijima Y, Matsu-ura Y, Urade Y, Hayaishi O. Expression of lipocalin-type prostaglandin D synthase (beta-trace) in human heart and its accumulation in the coronary circulation of angina patients. *Proc Natl Acad Sci U S A* 1997; 94:14689-94.

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