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Research letter

Serum soluble TREM2 is a potential novel biomarker of cognitive impairment in Japanese non-obese patients with diabetes

Introduction

The incidence of dementia has increased worldwide, and recent studies have reported diabetes as a risk factor for dementia, including Alzheimer's disease (AD) and vascular dementia (VaD) [1]. However, the detailed pathophysiology of dementia remains unclear. While obesity has been implicated in dementia, its role is controversial [2]. Also, predictive markers and effective treatment for dementia have yet to be established.

The triggering receptor expressed on myeloid cells 2 (TREM2) is a transmembrane protein mainly expressed on myeloid cells, such as macrophages (Mϕ) and microglia, and involved in inflammation [3]. Previous studies have suggested the pathological implications of TREM2 in neurodegenerative diseases, including AD [3]. TREM2 releases its ectodomain as a soluble form (sTREM2) into the extracellular space upon protease-mediated shedding [3,4]. Although the pathophysiological roles of sTREM2 in blood are still unclear [4,5], sTREM2 levels in cerebrospinal fluid (CSF) are elevated in subjects with AD dementia [5] or mild cognitive impairment (MCI) due to AD [6], suggesting that CSF sTREM2 reflects microglial activation in AD [5,6]. Furthermore, TREM2 expression is elevated in adipose tissue infiltrated by Mϕ in insulin-resistant/diabetic *db*/*db* mice [7], suggesting the involvement of TREM2 and/or sTREM2 in the diabetes phenotype and/or complications. Nevertheless, the pathophysiological roles of TREM2 and sTREM2 in human diabetes and obesity have yet to be elucidated. For this reason, the present study has examined the pathophysiological significance of serum sTREM2 in diabetes patients with and without obesity in a cross-sectional manner.

Methods

Subjects and study design

A total of 321 Japanese patients with diabetes were enrolled in a multicentre study—the Japan Obesity and Metabolic Syndrome Study (JOMS)/Japan Diabetes and Obesity Study 2 (J-DOS2)—which included 210 subjects whose serum sTREM2 levels were measured (Fig. S1; see supplementary materials associated with this article online). Also recruited were patients with type 2 diabetes (T2D), as defined by guidelines of the Japan Diabetes Society, while obese patients were defined as those with a body mass index (BMI) ≥ 25 kg/m^2, based on the guidelines of the Japan Society for the Study of Obesity (JASSO). Candidates were aged 20–79 years at enrolment, and were not preselected by cognition and mentation. Exclusion criteria were a previous history of cardiovascular or other vascular diseases, severe renal disease, severe liver dysfunction and secondary obesity because of endocrine disorders.

The present study was designed to examine the relationship between serum sTREM2 levels and the primary outcome—cognitive function as assessed by the Mini-Mental State Examination (MMSE)—in diabetes patients in a cross-sectional manner. The study protocol was approved by the Central Ethics Committee for Clinical Research at the National Hospital Organization headquarters (approval number 14-034), UMIN-CTR ID: UMIN000017929. Written informed consent was obtained from all participants.

Measurement of cognitive function

Cognitive function was assessed using the Japanese version of the MMSE (Nihon Bunka Kagakusha Co. Ltd., Tokyo; provided by Psychological Assessment Resources, Inc., Lutz, FL, USA) comprising questions on cognition (score range: 0–30; higher scores indicate better function) [8–10]. In the present study, MMSE scores ≤ 26 corresponded to a cognitively impaired group (MCI and dementia) [11], whereas scores ≤ 23 corresponded to dementia, according to the guidelines of six societies in Japan.

Data collection and laboratory methods

Anthropometric/metabolic parameters were measured according to the standard procedures [12]. Serum sTREM2 levels were quantified using Human TREM2 ELISA kits (RayBiotech, Norcross, GA, USA). Samples from different groups were randomized across the ELISA plates.

Statistical analysis

Data are presented as means ± SD or as medians and interquartile range (IQR). Unpaired *t*, Mann–Whitney *U* and Fisher's exact tests were used for comparing numerical and categorical variables between groups. Spearman's rank correlation coefficient (ρ) was employed to investigate correlations of sTREM2 with anthropometric and metabolic parameters; Spearman's partial rank correlation coefficients (ρ_{pa}) were obtained using age and gender as control variables. Logistic regression analysis was used to calculate unadjusted and adjusted odds ratios (ORs) for cognitive decline (MMSE scores < 26 and < 23, respectively). Multivariate logistic regression analysis was used to adjust for age and gender, and multivariate linear regression analysis was used to examine the influence of variables on sTREM2 levels. When building this model, age, gender and factors with $P < 0.10$ on correlation analysis were entered into the multivariate analysis. Post-hoc power analysis was used to determine

Abbreviations: AD, Alzheimer's disease; CI, confidence interval; CSF, cerebrospinal fluid; HbA1c, haemoglobin A1c; MCI, mild cognitive impairment; Mϕ, macrophages; MMSE, Mini-Mental State Examination; OR, odds ratio; sTREM2, soluble triggering receptor expressed on myeloid cells 2; TREM2, triggering receptor expressed on myeloid cells 2; VaD, vascular dementia.

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the statistical power of the study's sample sizes; $P < 0.05$ was considered significant. All statistical analyses were performed using SPSS version 22.0 software (IBM Corp., Armonk, NY, USA).

Results

Baseline characteristics

Our study included 107 non-obese and 103 obese patients with diabetes, of whom 58 (38 non-obese and 20 obese) had MMSE scores ≤ 26. No significant differences were found in the glucose metabolism-related profiles or sTREM2 levels between diabetes patients with and without obesity (Table S1; see supplementary materials associated with this article online). However, MMSE scores were lower in the non-obese than in obese diabetes patients ($P = 0.006$).

Correlations of MMSE scores with clinical parameters and sTREM2 levels

MMSE scores and gender were associated with ageing and risk of developing VaD, respectively [1,9]. In addition, relationships with and without adjustment for age and gender were also examined. For the entire study group, but not in the non-obese and obese patients, MMSE scores revealed positive correlations with BMI ($\rho_{pa} = 0.183$, $P = 0.008$) and leptin levels ($\rho_{pa} = 0.172$, $P = 0.015$), but no significant correlations with sTREM2 levels after adjustments (Table S2; see supplementary materials associated with this article online).

Characteristics of non-obese and obese diabetes patients with MMSE scores ≤ or > 26

Patients with MMSE scores ≤ 26 were older than those with scores > 26 (non-obese, $P = 0.002$; obese, $P = 0.007$; Table S3; see supplementary materials associated with this article online). In non-obese diabetes patients, sTREM2 levels were higher in subjects with MMSE scores ≤ 26 than in those with scores > 26 ($P = 0.007$). In obese diabetes patients, systolic blood pressure (SBP), but not sTREM2 levels, was higher in those with MMSE scores ≤ 26 *vs.* those with scores > 26 ($P = 0.012$).

Associations between clinical characteristics, sTREM2 levels and risk of cognitive impairment

In non-obese diabetes patients, raised sTREM2 levels showed a significant association with the risk of low MMSE scores ≤ 26 before and even after adjusting for age and gender [before adjustment, OR: 1.55, 95% confidence interval (CI): 1.20–2.00, $P < 0.001$; after adjustment, OR: 1.39, 95% CI: 1.06–1.81, $P = 0.017$; Fig. 1 and Tables S4 and S5; see supplementary materials associated with this article online]. In obese diabetes patients, increases in SBP, but not in sTREM2 levels, was associated with the risk of cognitive impairment after adjustment (OR: 1.03, 95% CI: 1.01–1.06; $P = 0.015$).

Regarding the risk of low MMSE scores ≤ 23, raised fasting plasma glucose (FPG) levels showed a significant association even after adjustment in non-obese diabetes patients (OR: 1.33, 95% CI: 1.01–1.75; $P = 0.043$), whereas increased sTREM2 levels did not (Tables S6 and S7; see supplementary materials associated with this article online). In obese diabetes patients, no significant association was found.

Statistical power was calculated using a post-hoc power analysis for the logistic regression analysis to examine the association of sTREM2 (per 1000 ng/L) with the risk of MMSE scores ≤ 26. When the desired detected OR was 1.25, the statistical power was 0.838, thereby confirming that these sample sizes were sufficient.

Correlations between sTREM2 levels and clinical parameters

In non-obese diabetes patients, sTREM2 levels were positively correlated with HbA_{1c} ($\rho_{pa} = 0.238$, $P = 0.015$), triglycerides (TG; $\rho_{pa} = 0.250$, $P = 0.010$) and high-sensitivity C-reactive protein (hsCRP; $\rho_{pa} = 0.302$, $P = 0.002$), but negatively correlated with adiponectin levels ($\rho_{pa} = -0.274$, $P = 0.005$; Table 1; see supplementary materials associated with this article online). Further multivariate analyses using a general linear model for correlations with $P < 0.10$ (Table 1) demonstrated that, in non-obese diabetes patients, sTREM2 levels were positively correlated with HbA_{1c} (standardized regression coefficient: $\beta = 0.196$, $P = 0.031$) and hsCRP ($\beta = 0.191$, $P = 0.038$), but negatively correlated with adiponectin ($\beta = -0.191$, $P = 0.039$). In obese diabetes patients, sTREM2 levels were positively correlated with adiponectin levels ($\rho_{pa} = 0.239$, $P = 0.020$), but no other correlations were significant.

Discussion

The present study is the first to show that raised sTREM2 levels are associated with the risk of cognitive impairment in non-obese patients with diabetes. Such levels are further revealed to be positively correlated with hyperglycaemia and exacerbation of inflammation, which are risk factors for dementia [13]. Accordingly,

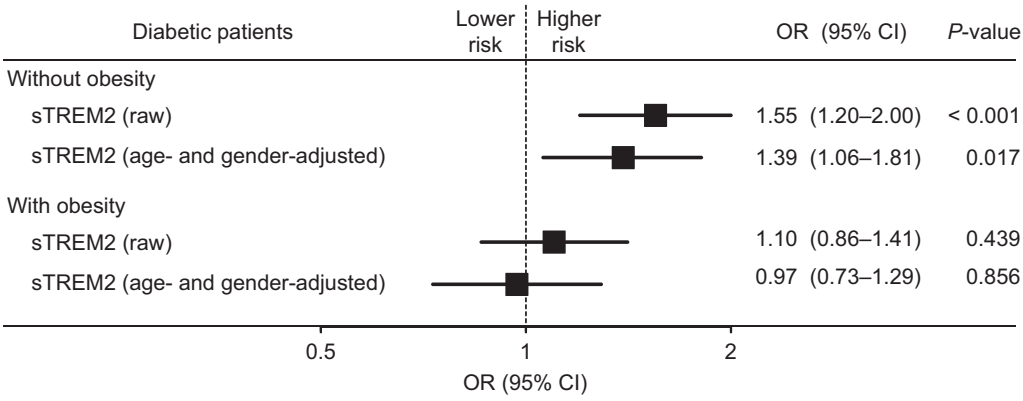

Fig. 1. Association between sTREM2 levels and risk of low MMSE scores (< 26) in diabetes patients. A total of 107 non-obese and 103 obese diabetes patients were examined to determine the association between sTREM2 levels (per 1000 ng/L) and risk of cognitive impairment, using univariate and multivariate logistic regression analyses. OR: odds ratio; CI: confidence interval.

Table 1
Age- and gender-adjusted correlations between soluble TREM2 levels and clinical characteristics in non-obese and obese patients with diabetes.

	Total ($n = 210$)		Without obesity ($n = 107$)		With obesity ($n = 103$)	
	ρ_{pa}[a]	P	ρ_{pa}[a]	P	ρ_{pa}[a]	P
Body mass index	0.021	0.761	−0.059	0.553	0.059	0.557
Systolic blood pressure	0.118	0.089	0.162	0.101	0.080	0.426
Diastolic blood pressure	−0.021	0.761	−0.003	0.977	−0.024	0.815
Fasting plasma glucose	0.059	0.396	−0.022	0.826	0.144	0.150
HbA_{1c}	0.194	0.005	0.238	0.015	0.149	0.138
Immunoreactive insulin	0.118	0.157	0.145	0.216	0.073	0.553
HOMA-IR	0.130	0.121	0.174	0.142	0.071	0.560
Total cholesterol	0.170	0.014	0.152	0.122	0.179	0.073
Triglycerides	0.195	0.005	0.250	0.010	0.158	0.115
HDL-C	0.016	0.816	−0.037	0.710	0.068	0.501
LDL-C	0.130	0.062	0.115	0.244	0.131	0.190
Leptin	0.126	0.077	0.141	0.160	0.109	0.292
Adiponectin	−0.065	0.360	−0.274	0.005	0.239	0.020
hsCRP	0.275	< 0.001	0.302	0.002	0.183	0.075
Taking antidiabetic drug	0.046	0.513	−0.104	0.293	0.179	0.072
Taking antihypertensive drug	−0.066	0.344	−0.019	0.844	−0.129	0.199
Taking antidyslipidaemic drug	−0.087	0.213	−0.094	0.338	−0.083	0.409

HOMA-IR: homoeostasis model assessment of insulin resistance; HDL-C/LDL-C: high-density lipoprotein/low-density lipoprotein cholesterol; hsCRP: high-sensitivity C-reactive protein.
[a] Spearman's partial rank correlation coefficient.

elevated sTREM2 levels indicate diabetes-related cognitive impairment in non-obese diabetes patients, further suggesting that serum sTREM2 levels may be a novel marker of subtle/early-stage cognitive impairment in such patients.

Although the pathological role of obesity in dementia remains inconclusive [2], these data on the relationships between MMSE scores, BMI and leptin levels appear to suggest a beneficial role for obesity-related increases in leptin in cognitive function in diabetes patients.

Our correlation profiles further suggest that the pathophysiological role of sTREM2 may differ between non-obese and obese patients with diabetes. As sTREM2-correlated hyperglycaemia and inflammation are factors inducing neuronal injury, some portion of serum sTREM2 in non-obese diabetics might be derived from activated microglia crossing the blood–brain barrier, the integrity of which is decreased in diabetes [13]. In obese diabetics, such correlations were not found, although some portion of sTREM2 may have originated from accumulations of MΦ in adipose tissue [7]. Furthermore, various potential molecular modifications of sTREM2, such as glycosylation, may be implicated in the differences in sTREM2 function in diabetes patients with and without obesity [4].

This study has some limitations. Because it is a cross-sectional study, it is difficult to conclude whether there is a causal relationship between sTREM2 and cognitive dysfunction. The function of sTREM2 in diabetes patients also remains unclear, although TREM2 is involved in adipogenesis, and sTREM2 triggers inflammation in mice [14,15]. In addition to a cohort study including healthy control subjects and patients with AD, other longitudinal prospective cohort studies with larger sample sizes, as well as experimental studies, are also required to elucidate the roles of sTREM2 in cognitive function in patients with diabetes.

In conclusion, our present findings suggest the usefulness of serum sTREM2 as a marker for the prevention and early management of hyperglycaemia and exacerbation of inflammation to reduce the risk of cognitive dysfunction in non-obese diabetes patients. Early control of SBP may also be significant for preventing cognitive impairment in obese diabetics. Further basic and clinical research is now required to corroborate our findings, and elucidate the mechanisms underlying the relationship between sTREM2 and cognitive dysfunction in diabetes patients, thereby contributing to preventative medicine for dementia.

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Authors' contributions

M.T. and H.Y. researched the data, contributed to the interpretation of findings and wrote, reviewed/edited the manuscript. S.M., T.I. and R.O.-K. researched the data, contributed to the interpretation of findings and reviewed/edited the manuscript. R.A., Y.M., M.S., T.N., K.Y., T.T., M.S., M.S. and M.N. contributed to the data collection and interpretation of data, and were involved in the drafting of the manuscript for important intellectual content. S.O., H.W., K.K. and T.K. contributed to the interpretation of findings, and reviewed the manuscript. A.S. contributed to the interpretation of findings and reviewed/edited the manuscript. K.H. contributed to the interpretation of findings, and wrote and reviewed/edited the manuscript. N.S.-A. researched the data, contributed to the interpretation of findings, and wrote and reviewed/edited the manuscript. All authors read and approved the final version of the manuscript.

Disclosure of interest

The authors declare that they have no competing interest.

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Appendix A. Supplementary data

Supplementary data (Fig. S1 and Tables S1–S7) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.diabet.2017.06.006.

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