Tissue inhibitor matrix metalloproteinase 1 and risk of type 2 diabetes in a Chinese population

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ABSTRACT

Introduction The non-invasive enhanced liver fibrosis (ELF) score—comprising tissue inhibitor of matrix metalloproteinases-1 (TIMP1), hyaluronic acid (HA) and amino-terminal propeptide of type III procollagen (PIIINP)—has been shown to accurately predict fibrosis stages among patients with non-alcoholic fatty liver disease (NAFLD). However, no study has examined whether the ELF score or its components would also be predictive of type 2 diabetes, which commonly coexists and shares the same pathogenic abnormalities with NAFLD. Therefore, we prospectively investigated their associations with type 2 diabetes risks for the first time.

Research design and methods The ELF score was measured among 254 type 2 diabetes cases and 254 age-matched and sex-matched controls nested within the prospective Singapore Chinese Health Study. Cases had hemoglobin A1c (HbA1c) levels <6.5% at blood collection (1999–2004) and reported to have diabetes during follow-up II (2006–2010). Controls had HbA1c levels <6.0% at blood-taking and remained free of diabetes at follow-up II. Multivariable conditional logistic regression models were used to assess the ELF-diabetes association.

Results Higher TIMP1 levels were associated with increased type 2 diabetes risk, and the OR comparing the highest versus lowest quartiles was 2.56 (95% CI 1.23 to 5.34; p trend=0.035). However, ELF score, PIIIMP and HA were not significantly associated with type 2 diabetes risks.

Conclusions Higher TIMP1 levels, but not ELF score, PIIIMP and HA, were associated with increased type 2 diabetes risk in Chinese adults. Our results suggested that elevated TIMP1 levels may contribute to the type 2 diabetes development through pathways other than liver fibrosis.

INTRODUCTION

Liver, a vital organ in maintaining glucose homeostasis, has been suggested to play an important role in the development of type 2 diabetes (T2D).1 The non-invasive enhanced liver fibrosis (ELF) score—comprising tissue inhibitor of matrix metalloproteinases-1 (TIMP1), hyaluronic acid (HA) and amino-terminal propeptide of type III procollagen (PIIINP)—has been shown to accurately diagnose advanced liver fibrosis2 and predict fibrosis stages3 among patients with non-alcoholic fatty liver disease (NAFLD). The National Institute for Health and Care Excellence recently recommended to use the ELF score to test for and monitor advanced liver fibrosis in people diagnosed with NAFLD.4 Since NAFLD and T2D commonly coexist and share the pathogenic abnormalities of excess adiposity and insulin resistance,5 we aimed to examine the prospective association between ELF score and incident T2D risk for the first time.

METHODS

The Singapore Chinese Health Study recruited 63,257 Chinese men and women (aged 45–74 years) with informed consent...
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between 1993 and 1998. At baseline, a structured questionnaire was used during a face-to-face interview to collect information on medical history, diet and lifestyle habits. Blood specimens were collected from 32,355 participants at follow-up I (1999–2004), and serum was extracted and stored at −80°C. After a mean follow-up of 4 years, 292 participants with hemoglobin A1c (HbA1c) levels <6.5% at blood-taking and free of diabetes reported to have physician-diagnosed diabetes during follow-up II (2006–2010), and were designated as incident T2D cases. Controls were randomly selected from those who had donated blood specimens, had HbA1c levels <6.0% at blood-taking and remained free of diabetes at follow-up II. Cases and controls were matched on a 1:1 ratio for age (±3 years), sex (men, women), date of blood collection (±6 months) and dialect group (Cantonese, Hokkien). After excluding those with insufficient serum sample (n=37) or extreme ELF score (>3 SD, n=1), 254 case-control pairs were included for the current analysis. Informed consent was provided and completed at the baseline interview.

Using the stored serum, the ELF score was measured from the same case-control pair in the same batch, using two-site sandwich assays (Siemens ADVIA Centaur XP system) at the National University Hospital Reference Laboratory, Singapore. In terms of other blood biomarkers, gamma-glutamyl transferase and alanine aminotransferase were measured using enzymatic method (Beckman Coulter, USA). High-density lipoprotein cholesterol, triglycerides and high-sensitivity C reactive protein were assayed by colorimetric method (Beckman Coulter). In addition, adiponectin was measured using sandwich ELISA method and HbA1c by cation exchange HPLC (Bio-Rad Laboratories, USA). The ELF score was calculated using the following equation: ELF score = 2.278 + 0.851 × ln(HA) + 0.751 × ln(nPIIINP) + 0.394 × ln(TIMP1). The levels of ELF score and three components were expressed as median (range).

Multivariable conditional logistic regression models were used to compute the OR and corresponding 95% CI for the associations between ELF score and each component and T2D risks. The final model was adjusted for age at blood-taking (years), education level (primary school and below, secondary or above), fasting status (yes, no), smoking (never, ever smoker), alcohol intake (never, ever drinker), weekly activity (<0.5, ≥0.5 hours/week), history of hypertension (yes, no), body mass index (kg/m²), triglycerides, high-sensitivity C reactive protein, high-density lipoprotein cholesterol, adiponectin, gamma-glutamyl transferase and alanine aminotransferase (all in quartiles). The blood biomarkers included in the final model have previously shown to be associated with incident T2D in this population. Potential interaction with age (<60 vs ≥60 years), sex, body mass index (<25 vs ≥25 kg/m²), alcohol consumption (never vs weekly or daily), fasting status and all blood biomarkers (<median vs ≥median levels) were also tested.

**Table 1** Baseline characteristics of incident type 2 diabetes cases and matched controls, the Singapore Chinese Health Study*

|                      | Cases (n=254) | Controls (n=254) | P value† |
|----------------------|--------------|------------------|----------|
| Age (years) at blood taken | 59.0±5.83    | 59.3±6.03        | –        |
| Gender (female)       | 134 (52.8)   | 134 (52.8)       | –        |
| Dialect (%)           |              |                  |          |
| Cantonese             | 127 (50.0)   | 127 (50.0)       |          |
| Hokkien               | 127 (50.0)   | 127 (50.0)       |          |
| Body mass index (kg/m²) | 24.8±3.68   | 22.6±3.41        | <0.001   |
| Level of education (%) |              |                  | 0.84     |
| No formal education   | 31 (12.2)    | 34 (13.4)        |          |
| Primary school        | 117 (46.1)   | 111 (43.7)       |          |
| Secondary and above   | 106 (41.7)   | 109 (42.9)       |          |
| History of hypertension (%) | 127 (50.0)  | 57 (22.4)        | <0.001  |
| Cigarette smoking (%) |              |                  | 0.57     |
| Never smokers         | 177 (70.0)   | 178 (70.1)       |          |
| Former smoker         | 34 (13.4)    | 40 (15.8)        |          |
| Current smokers       | 43 (16.9)    | 36 (14.2)        |          |
| Weekly moderate-to-vigorous activity (%) | 0.004      |                  |          |
| <0.5 hours/week       | 199 (78.4)   | 196 (77.2)       |          |
| 0.5–3.9 hours/week    | 44 (17.3)    | 29 (11.4)        |          |
| ≥4 hours/week         | 11 (4.3)     | 29 (11.4)        |          |
| Alcohol intake (%)    |              |                  | 0.95     |
| Abstainers            | 223 (87.8)   | 223 (87.8)       |          |
| Weekly drinkers       | 25 (9.8)     | 24 (9.5)         |          |
| Daily drinkers        | 6 (2.4)      | 7 (2.8)          |          |
| Fasting status (yes)  | 75 (29.5)    | 68 (26.8)        | 0.49     |
| ELF score, µg/L       | 9.2±8.71     | 9.1±0.69         | 0.12     |
| TIMP1, µg/L           | 227.9±39.0   | 213.5±35.6       | <0.001   |
| PIIINP, µg/L          | 7.56±2.69    | 7.01±2.75        | 0.023    |
| HA, µg/L              | 69.8±67.0    | 68.8±76.8        | 0.90     |
| GGT, IU/L             | 31 (21–46)   | 23 (17–35)       | <0.001   |
| ALT, IU/L             | 25 (19–36)   | 20 (15–27)       | <0.001   |
| HDL-C, mmol/L         | 1.08±0.26    | 1.23±0.31        | <0.001   |
| TG, mmol/L            | 2.1 (1.4–2.8)| 1.5 (1.0–2.0)    | <0.001   |
| Adiponectin, µg/mL    | 7.2±3.01     | 9.0±3.57         | <0.001   |
| hs-CRP, mg/L          | 1.6 (0.9–3.2)| 1.2 (0.5–2.1)    | <0.001   |
| HbA1c, %              | 5.9±0.35     | 5.5±0.27         | <0.001   |

*Data are expressed as mean±SD for continuous variables (normally distributed) and median (IQR) for continuous variables (skewed distributed), and n (percentage) for categorical variables. Cases and controls are matched on age at blood taken (±3 years), gender, dialect and date of blood collection (±6 months).

†P values based on the χ² test for categorical variables, student’s t-test for continuous variable with normal distribution and Mann-Whitney U test for continuous variable with skewed distribution.

RESULTS

Baseline characteristics of cases and controls are shown in table 1. Among the 254 T2D cases, the mean duration of hypertension (yes, no), body mass index (kg/m²), alcohol consumption (never vs weekly or daily), fasting status and all blood biomarkers (<median vs ≥median levels) were also tested.
Table 2. ORs (95% CIs) of type 2 diabetes associated with quartiles of ELF score, TIMP1, PIIINP and HA, the Singapore Chinese Health Study

| Variables                  | Quartiles of variables | ORs (95% CIs) | P for trend* | Per SD increment |
|----------------------------|------------------------|---------------|--------------|-----------------|
| ELF score†                 | Q1 (8.51–8.75)         | 1.00          | 0.88 (0.51 to 1.49) | 0.87 (0.49 to 1.54) | 1.54 (0.90 to 2.61) | 0.08 1.19 (0.98 to 1.45) | 0.94 1.03 (0.83 to 1.29) | 0.46 0.92 (0.71 to 1.20) |
| Case/controls              | 63/64                  | 53/63         | 50/64        | 88/63           |
| Model 1‡                   | 1.00                   | 0.87 (0.47 to 1.60) | 0.72 (0.37 to 1.39) | 1.03 (0.56 to 1.92) | 0.94 1.03 (0.83 to 1.29) | 0.46 0.92 (0.71 to 1.20) |
| Model 2§                   | 1.00                   | 0.88 (0.44 to 1.76) | 0.76 (0.37 to 1.56) | 0.79 (0.38 to 1.62) | 0.46 0.92 (0.71 to 1.20) |
| Model 3¶                   | 1.00                   | 0.88 (0.44 to 1.76) | 0.76 (0.37 to 1.56) | 0.79 (0.38 to 1.62) | 0.46 0.92 (0.71 to 1.20) |
| TIMP1, µg/L                | Median (176–186)       | 199 (187–209) | 221 (210–235) | 258 (236–406) |
| Case/controls              | 32/64                  | 56/63         | 66/64        | 100/63          |
| Model 1‡                   | 1.00                   | 1.85 (1.05 to 3.27) | 2.08 (1.17 to 3.68) | 3.02 (1.75 to 5.23) | <0.001 1.46 (1.21 to 1.76) |
| Model 2§                   | 1.00                   | 2.21 (1.16 to 4.22) | 2.09 (1.10 to 3.96) | 2.76 (1.46 to 5.21) | 0.006 1.42 (1.14 to 1.77) |
| Model 3¶                   | 1.00                   | 2.38 (1.15 to 4.90) | 2.27 (1.09 to 4.75) | 2.56 (1.23 to 5.34) | 0.035 1.36 (1.06 to 1.75) |
| PIIINP, µg/L               | Median (4.95–5.40)     | 5.90 (5.50–6.30) | 7.00 (6.40–7.90) | 9.60 (8.00–28.5) |
| Case/controls              | 48/70                  | 44/58         | 77/64        | 85/62           |
| Model 1‡                   | 1.00                   | 1.16 (0.66 to 2.05) | 1.92 (1.13 to 3.27) | 2.08 (1.24 to 3.48) | 0.001 1.21 (1.01 to 1.46) |
| Model 2§                   | 1.00                   | 1.03 (0.54 to 1.95) | 1.20 (0.65 to 2.20) | 1.50 (0.82 to 2.74) | 0.15 1.08 (0.88 to 1.33) |
| Model 3¶                   | 1.00                   | 0.92 (0.45 to 1.88) | 0.87 (0.43 to 1.75) | 1.19 (0.59 to 2.41) | 0.57 0.99 (0.79 to 1.25) |
| HA, µg/L                   | Median (26.2–33.2)     | 41.9 (33.4–50.1) | 60.1 (50.4–76.3) | 111 (76.4–936) |
| Case/controls              | 70/64                  | 52/63         | 61/64        | 71/63           |
| Model 1‡                   | 1.00                   | 0.74 (0.44 to 1.27) | 0.90 (0.52 to 1.56) | 1.09 (0.64 to 1.84) | 0.57 1.03 (0.86 to 1.25) |
| Model 2§                   | 1.00                   | 0.70 (0.37 to 1.32) | 0.93 (0.49 to 1.76) | 0.82 (0.44 to 1.54) | 0.79 0.95 (0.77 to 1.18) |
| Model 3¶                   | 1.00                   | 0.56 (0.27 to 1.15) | 0.90 (0.43 to 1.88) | 0.82 (0.30 to 1.27) | 0.42 0.87 (0.65 to 1.15) |

*Linear trend was tested using the quartiles of ELF score, TIMP1, PIIINP and HA as continuous variables.
†ELF score=2.278+0.851×ln(HA concentration)+0.751×ln(PIIINP concentration)+0.394×ln(TIMP1 concentration).
‡Model 1: adjusted for age at blood-taking (years), education level (primary school and below, secondary or above) and fasting status (yes, no).
§Model 2: model 1 plus smoking (never, ever smoker), alcohol intake (never, ever drinker), weekly activity (<0.5, ≥0.5 hours/week), history of hypertension (yes, no) and body mass index (kg/m²).
¶Model 3: model 2 plus triglycerides, high-density lipoprotein cholesterol, high-sensitivity C reactive protein, adiponectin, gamma-glutamyl transferase and alanine aminotransferase (all in quartiles).

ELF, enhanced liver fibrosis; HA, hyaluronic acid; PIIINP, amino-terminal propeptide of type III procollagen; Q, quartile; TIMP1, tissue inhibitor of matrix metalloproteinase 1.
biomarkers, the positive association was slightly attenuated but remained statistically significant (OR comparing extreme quartiles of TIMP1: 2.56 (95% CI 1.23 to 5.34; p trend=0.055)). The restricted cubic spline analysis suggested that the TIMP1-T2D association was linear (p for non-linearity=0.21) (figure 1). The OR associated with per SD increment of TIMP1 levels for T2D risk was 1.36 (95% CI 1.06 to 1.75) in the final model (table 2). Furthermore, in stratified analyses by baseline characteristics and other biomarkers, the TIMP1-T2D association was similar in the subgroups without significant interactions (data not shown).

**DISCUSSION**

In this prospective case-control study nested in a population-based cohort of middle-aged and older Chinese adults in Singapore, we did not find an association between the ELF score and T2D risk. Among the three components, TIMP1 levels were positively associated with diabetes risk; however, both PIIINP and HA were not associated with T2D risk. Therefore, elevated TIMP1 levels may contribute to the later development of incident T2D through pathways not greatly overlapping with liver fibrosis.

Our finding of the positive association between TIMP1 and T2D concur with results from several cross-sectional and case-control studies from Korea (cases n=80, controls n=80), Iraq (cases n=54, controls n=26), the UK (cases n=86, controls n=63) and the USA (n=1069). Although a case-control study in Greece (cases n=60, controls n=60) has observed lower TIMP1 levels in patients with T2D compared with controls, the heterogeneous results could be explained by the different characteristics of patients included in the study. Compared with all other studies, patients in the Greek study were at more advanced stage of T2D and may have received more intensive treatment; the intensified diabetes therapy have shown to decrease TIMP1 levels significantly, which may explain for the lower TIMP1 levels among patients with diabetes in the Greek study. For PIIINP and HA, evidence is scarce regarding their associations with T2D risks; therefore, further studies are warranted to validate our findings.

TIMP1 is a biomarker for systemic fibrosis. Excess deposition of extracellular matrix is the hallmark of systemic fibrosis, and TIMP1 promotes fibrosis by inhibiting metalloproteinases in the breakdown of extracellular matrix. In support of this, epidemiological findings have reported different levels of metalloproteinases, the target enzyme of TIMP1 inhibition, between people with and without T2D. TIMP1 also enhances adipogenesis by accelerating lipid accumulation and adipocyte differentiation, and this process results in the rapid expansion of adipose tissues, which in turn contributes to subsequent hypoxia and inflammation in the adipose tissues, and further aggravating adipose tissue fibrosis. Hence, TIMP1 could be implicated in the pathogenesis of T2D by its role in adiposity, systemic fibrosis and inflammation, which have all been shown to be associated with metabolic disturbances and resulting in insulin resistance.

To our best knowledge, this is the first prospective study investigating the association between ELF score and T2D risk. The exclusion of undiagnosed diabetes in cases at the time of blood-taking using HbA1c as diagnostic criteria is a strength of the current study in establishing temporality in the association. We also excluded possible undiagnosed diabetes among controls by using stringent HbA1c criteria at the time of blood-taking. However, several limitations merit consideration. First, residual confounding may exist since height and weight were self-reported in the current study, and some major T2D risk factors, such as family history of T2D, fasting levels of glucose and insulin and inflammatory markers (eg, IL-6), were not available in the current study due to lack of information. We did not collect the information on family history of T2D because the current cohort was initially established for cancer research. In addition, about two-thirds of the blood samples were non-fasted. However, we stratified the analysis by fasting status and observed similar TIMP1-T2D associations in both subgroups, suggesting that the fasting status is unlikely to affect the observed association. The current study was conducted among middle-aged and elderly population, and generalisability to younger population is a concern, although no significant interaction was found with age. Furthermore, the current study had a relatively short follow-up time of about 4 years; future studies with longer follow-up durations are warranted to examine the predictive performance of TIMP1 for the long-term T2D risk.
In conclusion, in this case-control study nested within a prospective cohort among Chinese living in Singapore, we found no significant association between the ELF score and T2D risk, but a positive association between TIMP1 and T2D risk. Our results suggested that elevated TIMP1 levels may contribute to the T2D development through pathways other than liver fibrosis. Future studies are warranted to validate the current finding and to elucidate the underlying mechanism to facilitate the development of drugs and therapies targeting TIMP1 levels for the prevention and management of T2D risk.

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Contributors AP and W-PK conceived the study, interpreted the data and critically revised the reports. YW did the search, analyzed and interpreted the data, drafted and critically revised the reports. J-MY contributed to the acquisition of study materials and critically revised the reports. All authors revised and approved the final report.

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