Causal association pathways between fetuin-A and kidney function: A mediation Analysis

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Abstract

Background

Body mass index (BMI), uric acid (UA) diabetes mellitus (DM) and hypertension (HT) are known risk factors of declined kidney function, and are associated with fetuin-A. However, the causal pathways of these associations are unclear. We therefore used cohort data to explore possible causal pathways of fetuin-A and kidney function.

Methodology

We used data of the Electricity Generating Authority of Thailand cohort 2009 (n= 2305). A causal pathway was constructed, which considered fetuin-A as study factor, BMI, UA, DM, and HT as mediators, and eGFR as the outcome. A generalized-structural equation model (GSEM) with 1000-replication bootstrapping was applied to assess the causal effects adjusting for covariates.

Results

The fetuin-A → eGFR pathway showed a direct association of fetuin-A on eGFR with the coefficient of -0.0072 (95% CI: -0.0119, -0.0025). In addition, the indirect effects of fetuin-A→ BMI → eGFR was also significant with the coefficient of 0.00086 (0.00025, 0.0016; implying that every one unit of BMI increased, resulting from increasing fetuin-A, would significantly increase eGFR 0.00086 (0.00025, 0.0016) mL/min/1.73m². There was a negative effect of fetuin-A on eGFR through BMI and UA pathway (Fetuin-A→BMI→UA→eGFR ) as well as the HT pathway (Fetuin-A→BMI→HT→eGFR ) with average casual mediation effects (ACME) of -0.00132 (-0.00177, -0.00092) and -0.00139 (-0.00237, -0.00069). Fetuin-AàDMàHTàeGFR was also statistically significant with the ACME of -0.00223 (-0.00535, -0.00066).

Conclusion

Our study has shed some light on the possible role of fetuin-A in the etiology of declining renal function through the mediatory roles of BMI, UA, DM and HT in the various complex causal pathways leading to declining kidney function in our study cohort. Further studies are however recommended to examine the pathomechanisms involved in the mediational processes of these studied risk factors in the etiology of declining kidney function.

Background

Chronic kidney disease (CKD) is a global public health problem with rising numbers of renal failure(1, 2). The report of the 2015 Global Burden of Disease Study indicated that kidney disease was the 12th most common cause of death, which accounted for 1.1 million deaths worldwide(1). Known risk factors for declined kidney function have been reported including body mass index (BMI)(3, 4), uric acid (UA)(5, 6), diabetes mellitus (DM)(7–9) and hypertension (HT)(10, 11). Some evidences have also shown that inflammatory markers (e.g., high sensitivity C-Reactive protein (hsCRP), interleukin-1 (IL-1), endothelin-1
(ET -1), and tumor necrosis factor-α (TNF-α) (12, 13) are associated with eGFR decline. In addition, recent evidences from observational studies showed that fetuin-A was also associated with CKD morbidity (14, 15) and mortality (16, 17). Moreover, fetuin-A has been found to be associated with CKD risk including BMI (18), DM (19, 20) and HT (21, 22). However, the causal pathways of these disease phenotypes are quite complex and not fully understood. Fetuin-A might be directly associated with declined eGFR, or it might affect eGFR through mediators (i.e., BMI, UA, DM, and HT) which are also individually associated with declining eGFR.

Considering the fact that fetuin-A, a pleiotropic multi-functional circulating glycoprotein, had been found to be associated with the afore-mentioned disease phenotypes, we therefore conducted a study using data from a prospective cohort of the Electricity Generating Authority of Thailand (EGAT) to explore the causal association pathways of these diseases with fetuin-A in relation to declining kidney function.

**Design**

We used cross-sectional data of EGAT prospective cohort (23), who were recruited in 2009 and followed up in 2014 (5 years later) with a sample sizes of 2,564. The cohort was originally designed to assess risk factors for cardiovascular diseases, psychological distress, health status, functional status and health-related quality of life. All EGAT’s employees aged 18 years or older were invited to voluntarily participate in this cohort study after obtaining their written informed consent. At inception, the subjects underwent medical examinations including laboratory tests and also completed a self-administered questionnaire on their lifestyle behavior and family history of disease. Members of the respective cohorts are resurveyed regularly every 5 years.

The main study factor was serum fetuin-A, which was measured using specimens that were collected at the baseline survey in 2009 by sandwich enzyme immunoassay (R&D Systems, Inc., Minneapolis, MN, USA). Precisions of intra- and inter-assays were 4.9% and 7.3%, respectively (18). Our interested clinical outcome was estimated eGFR which was estimated based on the CKD-EPI Creatinine Eq. (2009) (24) using serum creatinine, age and sex parameters. In addition, we also had intermediate outcomes, which were considered as mediators, including BMI, UA, DM, and HT. The outcome and mediators were measured at survey 2009 and 2014. BMI was calculated as weight (kg)/height (m²). UA was assessed by the spectrophotometric absorption technique after treatment of the specimen with the enzyme uricase. Then, it was classified as normal if the value was in the range 2.5 to 7.5 milligrams /deciliter (mg/dL) for women and 4.0 to 8.5 mg/d for men (25), otherwise it was abnormal. DM was diagnosed if fasting blood sugar (FBS) was ≥ 7.0 mmol/l (or 126 mg/dl) with/without a history or evidence of use of anti-diabetic medication (26). Subjects were classified as HT if they took any of antihypertensive drugs (e.g., calcium channel blockers, beta-blocker, angiotensin converting enzyme (ace) inhibitors, and etc.), or had systolic blood pressure (SBP) or diastolic blood pressure (DBP) ≥ 140 and ≥ 90 mmHg, respectively. Blood pressure was measured twice; taken 5 minutes apart and allowing for a 5-minutes rest after the first measurement. Covariables that were considered included demographic variables (i.e., age, gender),
smoking history (current/ex-smokers and non-smokers), alcohol consumption (current/ex-drinkers and non-drinker), triglyceride, and LDL.

**Statistical analysis**

Baseline and follow-up demographic variables were analyzed and reported as means ± SD for continuous data, frequency and percentage for categorical data. Multiple mediation analysis was performed according to the causal association pathways (See Fig. 1. Causal association pathways between fetuin-A and eGFR), in which fetuin-A was considered as independent variable, BMI, UA, DM, and HT were mediators, and eGFR was the outcome. All possible serial multiple mediation models were constructed, see Supplement Table 1. Mediation and outcome models were constructed by fitting fetuin-A on each of the four mediators (i.e., BMI, UA, DM, and HT) using generalized linear structural equation models (GSEM) with logit link for DM and HT, and identity link for BMI, UA, and eGFR.
| Characteristics                  | Survey 2009 | Survey 2014 |
|----------------------------------|-------------|-------------|
| Number of subjects               | 2564        | 2305        |
| Age, year, mean (SD)             | 41 ±7       | 46 ±7       |
| Gender, number (%)               |             |             |
| Male                             | 1882 (73.4) | 1656 (71.8) |
| Female                           | 682 (26.6)  | 649 (28.2)  |
| Marital Status, number (%)       |             |             |
| Single                           | 745 (29.1)  | 461 (20.0)  |
| Married                          | 1705 (66.5) | 1641 (71.2) |
| Divorced/Widowed                 | 114 (4.4)   | 203 (8.8)   |
| Income, Baht/month, number (%)   |             |             |
| <20,000                          | 130 (5.1)   | -           |
| 20,000–49,999                    | 970 (38.1)  | -           |
| ≥50,000                          | 1446 (56.8) | -           |
| Education, number (%)            |             |             |
| ≤Secondary school               | 115 (4.5)   | 75 (3.3)    |
| Vocational                       | 584 (22.8)  | 473 (20.5)  |
| ≥Bachelor                        | 1865 (72.7) | 1757 (76.2) |
| Smoking, number (%)              |             |             |
| Never smoker                     | 1683 (65.7) | 1499 (65.0) |
| Ex-smoker                        | 450 (17.6)  | 475 (20.6)  |
| Characteristics          | Survey 2009   | Survey 2014   |
|--------------------------|---------------|---------------|
| Current smoker           | 428 (16.7)    | 331 (14.4)    |
| Alcohol, number (%)     |               |               |
| Never Drinkers          | 981 (39.1)    | 906 (39.3)    |
| Quit drinkers            | 171 (6.8)     | -             |
| Current drinkers         | 1358 (54.1)   | 1399 (60.7)   |
| Exercise, times/week, number (%) |         |               |
| None                     | 1503 (58.7)   | 743 (32.2)    |
| 1–2                      | 406 (15.8)    | 446 (19.4)    |
| ≥3                       | 652 (25.5)    | 1116 (48.4)   |
| Diabetes                 |               |               |
| Yes                      | 117 (4.6)     | 236 (10.2)    |
| No                       | 2440 (95.4)   | 2069 (89.8)   |
| Hypertension             |               |               |
| Yes                      | 500 (22.6)    | 809 (35.1)    |
| No                       | 1984 (77.4)   | 1496 (64.9)   |
| BMI, kg/m\(^2\), mean (SD) | 24.0 ±3.7    | 24.8 ±3.8     |
| Fetuin, mg/dl, mean (SD) | 558.9 ±110.5  | -             |
| Uric Acid, mg/dl, mean (SD) | 5.6 ±1.5     | 5.8 ±1.5     |
| Cholesterol, mg/dl, mean (SD) | 216.8 ±39.3  | 217.4 ±40.3  |
| HDL, mg/dl, mean (SD)    | 51.7 ±12.3    | 58.3 ±15.5    |
| LDL, mg/dl, mean (SD)    | 148.4 ±36.9   | 148.6 ±37.7   |
| Triglyceride, mg/dl, mean (SD) | 129.1 ±90    | 131.8 ±84.1   |
| eGFR, ml/min/1.73 m\(^2\), mean (SD) | 98.7 ±23.6 | 92.4 ±22.9   |
A univariate GSEM model was used to screen the covariables (i.e., age, gender, smoking, alcohol, triglycerides, and LDL) that might associate with each of mediators (i.e., BMI, UA, DM, and HT). Forward selection was applied to select significant variables in the mediation and outcome models that already contained fetuin-A. Mediated effects were then estimated by product coefficients of each pathway, see Supplement Table 1. Finally, bias corrected bootstrap with 1000 replications was used to estimate average mediation effects(27). All analyses were performed using STATA(28) version 15, p-value of < 0.05 was considered statistically significant.

**Results**

Baseline characteristics are described (see Table 1), in which 2564 subjects were enrolled in 2009 but only 2305 subjects remained for follow up in the 2014 survey. Mean age, BMI, UA, and eGFR at baseline were respectively 46 ±7 years, 24.8 ±3.8 kg/m$^2$, 5.6 ±1.5, and 98.7 ±23.6, whereas the prevalence of DM and HT were 4.6% and 22.6%.

The univariate GSEM showed that fetuin-A significantly associated with all mediators (BMI, UA, DM, and HT) and eGFR outcome see Supplement Table 2. In addition, all covariables including age, sex, smoking, alcohol, triglyceride, and LDL were also significantly associated with each mediator and eGFR. Multivariate GSEMs were then constructed considering all 6 covariables for BMI, UA, DM, and HT and eGFR models, see Table 2. After adjusting covariables, fetuin-A was significantly associated with only mediator BMI and DM but not with UA and HT. In addition, fetuin-A was also significantly associated with eGFR.
### Table 2
Causal associations between fetuin-A and eGFR: Multivariate GSEM models

| Equation | Factors                  | b      | SE    | z     | p       | 95% CI       |
|----------|--------------------------|--------|-------|-------|---------|--------------|
| Fetuin→BMI | Fetuin                  | 0.0039 | 0.0006| 7.12  | < 0.001 | 0.0029, 0.0051 |
|          | Age                     | 0.0368 | 0.0088| 4.17  | < 0.001 | 0.0195, 0.0541 |
|          | Male vs. Female          | 1.4058 | 0.1549| 9.08  | < 0.001 | 1.1022, 1.7093 |
|          | Smoking                  |        |       |       |         |              |
|          | Ex-smoker                | 0.4306 | 0.1718| 2.51  | 0.012   | 0.0939, 0.7674 |
|          | Current smoker           | -0.3115| 0.1890| -1.65 | 0.099   | -0.6820, 0.0589 |
|          | Alcohol drinking         |        |       |       |         |              |
|          | Ex-drinker               | 0.5371 | 0.2417| 2.22  | 0.026   | 0.0634, 1.0108 |
|          | Current drinker          | 0.3329 | 0.1463| 2.28  | 0.023   | 0.0463, 0.6196 |
|          | Triglyceride             | 0.0109 | 0.0008| 14.19 | < 0.001 | 0.0094, 0.0124 |
|          | LDL                      | 0.0062 | 0.0017| 3.66  | < 0.001 | 0.0029, 0.0096 |
| Fetuin→Uric acid | Fetuin                  | 0.0002 | 0.0002| 1.29  | 0.196   | -0.0001, 0.0006 |
|          | BMI                      | 0.0849 | 0.0055| 15.51 | < 0.001 | 0.0742, 0.0957 |
|          | Male vs. Female          | 1.5952 | 0.0493| 32.33 | < 0.001 | 1.4984, 1.6919 |
|          | Smoking                  |        |       |       |         |              |
|          | Ex-smoker                | -0.0095| 0.0543| -0.17 | 0.862   | -0.1159, 0.0969 |
|          | Current smoker           | -0.1707| 0.0597| -2.86 | 0.004   | -0.2877, -0.0537 |
|          | Alcohol drinking         |        |       |       |         |              |
| Equation | Factors               | b      | SE     | z      | p       | 95% CI      |
|----------|-----------------------|--------|--------|--------|---------|-------------|
|          | Ex-drinker            | 0.0859 | 0.0764 | 1.13   | 0.261   | -0.0638, 0.2356 |
|          | Current drinker       | 0.2249 | 0.0462 | 4.87   | < 0.001 | 0.1343, 0.3155  |
|          | Triglyceride          | 0.0025 | 0.0002 | 10.19  | < 0.001 | 0.0020, 0.0030  |
| Fetuin   | Fetusin-A→DM          | 0.0015 | 0.0005 | 2.75   | 0.006   | 0.0004, 0.0026  |
|          | BMI                   | 0.1870 | 0.0159 | 11.71  | < 0.001 | 0.1557, 0.2183  |
|          | Uric acid             | -0.2350| 0.0527 | -4.46  | < 0.001 | -0.3383, -0.1317|
|          | Age                   | 0.0639 | 0.0092 | 6.96   | < 0.001 | 0.0459, 0.0820  |
|          | Male vs. Female       | 0.5310 | 0.1725 | 3.08   | 0.002   | 0.1930, 0.8690  |
|          | Triglyceride          | -0.0102| 0.0017 | -5.86  | < 0.001 | -0.0136, -0.0068|
|          | LDL                   | 0.0025 | 0.0006 | 4.03   | < 0.001 | 0.0013, 0.0037  |
| Fetusin-A→HT | Fetuin               | 0.0008 | 0.0004 | 1.89   | 0.059   | -0.00003, 0.0016 |
|          | BMI                   | 0.1389 | 0.0132 | 10.54  | < 0.001 | 0.1131, 0.1647  |
|          | Uric acid             | 0.1759 | 0.03333| 5.28   | < 0.001 | 0.1106, 0.2413  |
|          | DM                    | 0.5955 | 0.1338 | 4.45   | < 0.001 | 0.3333, 0.8576  |
|          | Age                   | 0.0919 | 0.0069 | 13.39  | < 0.001 | 0.0784, 0.1053  |
|          | Triglyceride          | -0.0066| 0.0013 | -5.28  | < 0.001 | -0.0091, -0.0042|
|          | LDL                   | 0.0031 | 0.0005 | 5.71   | < 0.001 | 0.0020, 0.0042  |
| Fetuin   | Fetuin                | -0.0077| 0.0022 | -3.49  | < 0.001 | -0.0119, -0.0034|

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A bootstrap with 1000-replication was applied to estimate average causal mediation effects (ACMEs), see Table 3. The result indicated that most fetuin-A effects on eGFR through BMI pathway were significant. For instance, ACME of Fetuin→BMI→eGFR pathway was 0.000864 (0.00025, 0.00163), i.e., increasing fetuin-A one unit would increase BMI and then increased eGFR of 0.000864 unit. Fetuin-A effects through BMI and then UA pathway (Fetuin→A→BMI→UA→eGFR ) and HT pathway (Fetuin-A→BMI→HT→eGFR ) showed significantly negative effects, i.e., lowering eGFR with ACMEs of -0.00132 (-0.00177, -0.00092) and -0.00139 (-0.00237, -0.00069). In addition, fetuin-A◊DM◊HT◊eGFR was also statistically significant with the ACME of -0.00223 (-0.00535, -0.00066). None of the other three single-mediator pathways: fetuin-A◊UA, fetuin-A◊DM, and fetuin-A◊HT, was statistically significant.
Table 3
Estimation of average causal mediation effects using bootstrapping

| Paths                        | Effect     | SE         | Z       | P-value | 95% CI       |
|------------------------------|------------|------------|---------|---------|--------------|
| Fetuin→eGFR (direct effect)  | -0.00721   | 0.00260    | -2.77   | 0.006   | -0.01190, -0.00247 |
| BMI-mediator                 |            |            |         |         |              |
| Fetuin→BMI→eGFR             | 0.000864   | 0.000353   | 2.45    | 0.014   | 0.00025, 0.00163 |
| Fetuin→BMI→U→eGFR           | -0.00132   | 0.00022    | -5.99   | <0.001  | -0.00177, -0.00092 |
| Fetuin→BMI→D→eGFR           | -0.000165  | 0.000674   | -0.24   | 0.807   | -0.00149, 0.00116 |
| Fetuin→BMI→H→eGFR           | -0.00139   | 0.000416   | -3.35   | 0.001   | -0.00237, -0.00069 |
| Fetuin→BMI→U→DM→eGFR        | 0.000018   | 0.000076   | 0.23    | 0.817   | -0.00005, 0.00028 |
| UA mediator                  |            |            |         |         |              |
| Fetuin→UA→eGFR              | -0.00089   | 0.00067    | -1.35   | 0.177   | -0.00216, 0.00039 |
| Fetuin→UA→DM→eGFR           | 0.00001    | 0.00007    | 0.17    | 0.863   | -0.00006, 0.00024 |
| Fetuin→UA→HT→eGFR           | -0.00010   | 0.00009    | -1.18   | 0.237   | -0.00031, 0.00003 |
| Fetuin→UA→DM→HT→eGFR        | 0.00008    | 0.00007    | 1.11    | 0.269   | -0.00002, 0.00032 |
| Paths | Effect  | SE      | Z   | P-value | 95% CI            |
|-------|---------|---------|-----|---------|-------------------|
| DM mediator |         |         |     |         |                   |
| Fetuin→DM→eGFR | -0.00033 | 0.00139 | -0.24 | 0.812   | -0.00332, 0.00230 |
| Fetuin→DM→HT→eGFR | -0.00223 | 0.00113 | -1.97 | 0.049   | -0.00535, -0.00066 |
| HT mediator |         |         |     |         |                   |
| Fetuin-A→HT→eGFR | -0.00192 | 0.00118 | -1.64 | 0.102   | -0.00459, 0.00008 |

**Discussion**

We had explored causal pathway of fetuin-A and kidney function through BMI, UA, DM, and HT using multiple mediation analysis. Our findings indicated that fetuin-A directly associated with decreasing eGFR. In addition, effects of fetuin-A on eGFR was found to pass through the following mediators: BMI, UA (fetuin-A→BMI→UA→eGFR) and HT (fetuin-A→BMI→HT→eGFR), i.e., every unit of fetuin-A increased would increase BMI and UA risk resulting in a decrease in eGFR of 0.00132 ml/min/1.73 m². Likewise, increasing fetuin-A would increase BMI, HT risk, and decrease eGFR by 0.00139 ml/min/1.73 m². Furthermore, effects of fetuin-A could be mediated through DM and HT resulting in the lowering of eGFR.

Our finding with regards to the association of fetuin-A level with eGFR is similar to a previous study by Ix et al(29), which showed negative association between fetuin-A and predominantly non-diabetic subjects with stage 3 or 4 CKD, i.e., high fetuin-A level and low eGFR. Contrastingly, previous observational studies(14, 15, 30) found positive association between fetuin-A and kidney function, i.e., decreasing serum fetuin-A would decrease eGFR. It is worth noting that the functions and regulatory mechanisms of fetuin-A are complex, and may seem to differ according to the pathophysiologic characteristics of the population being studied(14). Several studies have demonstrated that inflammatory processes are increased in CKD, even in the early stages of CKD, and that the inflammatory processes triggered by inflammatory markers such as CRP and adiponectin are linked to endothelial dysfunction(14, 31, 32).

Fetuin-A, an anti-inflammatory protein acts as a negative acute phase reactant in the extra-cellular space to attenuate inflammatory responses, as such in patients with less advanced stages of CKD, and likely in the early phase of inflammation, fetuin-A levels may be normal or raised, however its expression is negatively regulated by pro-inflammatory cytokines such as CRP, which downregulates its synthesis during inflammation.(33, 34) Therefore, in a sustained inflammatory response, circulating fetuin-A levels are progressively depleted and its protective role in halting further decline of kidney function is
undermined. Moreover, though not consistently demonstrated(16), variations in fetuin-A levels may also be determined by genetic polymorphisms independent of inflammation(15, 35).

Overweight/obesity are known risk factors of cardiovascular disease (CVD)(36) and declining kidney function(4, 37). In addition, overweight/obesity is also highly associated with other CVD risks such as UA(38), DM(39) and HT (40), which may impact on kidney function as demonstrated by our findings. However, our mediation analysis showed positive causal effect of fetuin-A on eGFR that was mediated through BMI, i.e., BMI was a protective factor on kidney function. This might imply that the kidney function of subjects with high BMI could still be in good condition, if there are no associated risk factors such as hyperuricemia, DM, and HT.

Diabetes and hypertension are comorbid conditions frequently associated with kidney functions(41, 42). The role of DM in the pathogenesis of kidney disease has been established by epidemiological studies(9). Older subjects with longer duration of DM have a higher risk of developing CKD(43) and about 40% of patients with DM develop impaired kidney function, albuminuria, or both(44). Common kidney diseases which are associated with DM include CKD, ischemic nephropathy related to diabetic vascular disease and hypertensive nephrosclerosis(45, 46).

Fetuin-A is secreted predominantly by the hepatocytes and is encoded by the alpha Heremans-Schmidt glycoprotein (AHSG) gene, which is located on chromosome (3q27)(47). Its physiologic role includes the regulation of bone metabolism and the inhibition of vascular calcification. It has been implicated in vascular inflammatory processes as well as in the etiology of complex diseases such as CVDs(48) and DM(19, 20). Though some studies have been conducted to assess the relation of fetuin-A to CKD morbidity and progression (14, 49), and mortality(30, 33, 50) however its role in the etiology of kidney disease remains unclear.

Some observational studies have shown that increasing fetuin-A levels is associated with both improvements in the CKD status(31, 33) and endothelial dysfunction (ED)(31). ED is considered to be one of the major causal pathomechanisms of CKD(14, 51). Additionally, ED has equally been implicated in the pathophysiology of different forms of complex phenotypes like hypertension / coronary artery disease(52), DM(53) and CKD(54), which might be associated with the ED vascular inflammatory processes.

Our study have some strength. We assessed not only the direct causal effect of fetuin-A on kidney function but also the effects that were mediated through known risk factors of kidney function including BMI, UA, DM, and HT. We applied a multiple-mediation analysis model to determine possible causal pathways and effects of fetuin-A on eGFR. We used the EGAT prospective cohort to demonstrate the causal pathways that fetuin-A could have on kidney function through multiple mediator pathways, adjusting for covariables, which were obtained during the baseline and follow-up visits. A few limitations should however be addressed. Although we used longitudinal data from the EGAT cohort, fetuin-A was measured only once at baseline because of budget limitation. We considered intermediate mediators and
also surrogate outcome of eGFR instead of end-clinical outcomes because the cohort had been followed up for only 5 years.

In conclusion, fetuin-A might have direct effect on declining kidney function, in which increasing fetuin-A might reduce kidney function. In addition, its effects might be mediated through multiple mediators including BMI, UA, DM, and HT, resulting in the lowering of eGFR. High level fetuin-A might increase BMI, however among this EGAT cohort that were studied, raised BMI on its own had no effect on declining kidney. The effect of raised BMI in declining kidney function was observed with the inclusion of other risk factors such as DM, HT, or high UA. These findings would however need to be further assessed in a cohort with a longer follow-up period.

**Abbreviations**

AHSG alpha Heremans-Schmidt glycoprotein  
BMI body mass index  
CKD chronic kidney disease  
CVD cardio vascular disease  
DBP diastolic blood pressure  
DM diabetes mellitus  
EGAT Electricity Generating Authority of Thailand  
eGFR estimated glomerular filtration rate  
ET endothelin  
GSEM generalized-structural equation model  
hsCRP high sensitivity C-Reactive Protein  
HT hypertension  
IL Interleukin  
SBP systolic blood pressure  
TNF tumor necrosis factor  
UA uric acid
Declarations

Ethics approval and consent to participate

The ethical approval of the EGAT cohort study was given by Faculty of Medicine Ramathibodi Hospital, Mahidol University.

Duly signed consent was obtained from all the subjects before they were recruited into the study after the benefits and possible harms of the study was explained to them. The anonymity of the subjects was also ensured.

Consent for publication

Not applicable.

Availability of data and materials

a. The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

All the authors declare that they have no competing interests in this study.

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None.

Authors' contributions

The data analysis was carried out by PEM and AT. The initial draft of the manuscript was done by PEMB while the proof-reading was done by AT, PN, PS and MM. Data validation and reference check was done by SR and PN. All authors read and approved the final manuscript.

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References
1. GBD 2015 Mortality and Causes of Death Collaborators. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet. 2016;388:1459–544.

2. Couser WG, Remuzzi G, Mendis S, Tonelli M. The contribution of chronic kidney disease to the global burden of major noncommunicable diseases. Kidney Int. 2011;80:1258–70.

3. Garofalo C, Borrelli S, Minutolo R, Chiodini P, De Nicola L, Conte G. A systematic review and meta-analysis suggests obesity predicts onset of chronic kidney disease in the general population; Kidney International. Kidney Int. 2017;91:1224–35.

4. Nomura I, Kato J, Kitamura K. Association between body mass index and chronic kidney disease: A population-based, cross-sectional study of a Japanese community. Vasc Health Risk Manag. 2009;5:315–20.

5. Obermayr R, Temml C, Gutjahr G, Knechtelsdorfer M, Oberbauer R, Klauser-Braun R. Elevated Uric Acid Increases the Risk for Kidney Disease. J Am Soc Nephrol. 2008;19(12):2407–13.

6. Zhou F, Yu G, Wang G, Liu Y, Zhang L, Wang W, et al. Association of serum uric acid levels with the incident of kidney disease and rapid eGFR decline in Chinese individuals with eGFR > 60 mL/min/1.73 m2 and negative proteinuria. CLIN EXP NEPHROL. 2019;23:871–9.

7. Kim K-S, Park SW, Cho Y-W, Kim S-K. Higher Prevalence and Progression Rate of Chronic Kidney Disease in Elderly Patients with Type 2 Diabetes Mellitus. Diabetes Metab J. 2018;42:224–32.

8. Ma RCW. Epidemiology of diabetes and diabetic complications in China. Diabetologia. 2018;61:1249–60.

9. Wu B, Bell K, Stanford A, Kern DM, Tunceli O, Vupputuri S, et al. Understanding CKD among patients with T2DM: prevalence, temporal trends, and treatment patterns—NHANES 2007–2012. BMJ Open Diabetes Res Care. 2016;4(1):e000154.

10. Zhang a Y-P, Zuob X-C, Huangb Z-J, Kuanga Z-M, Luc M-G, Duand D, et al. The Impact of Blood Pressure on Kidney Function in the Elderly: A Cross-Sectional Study. Kidney Blood Press Res. 2013;38:205–16.

11. Yu Z, Rebholz C, Wong E, Chen Y, Matsushita K, Coresh J, et al. Association Between Hypertension and Kidney Function Decline: The Atherosclerosis Risk in Communities (ARIC) Study. AJKD. 2019;74(3):310–9.

12. Shankar A, Sun L, Klein BEK, Lee KE, Muntner P, Nieto FJ, et al. Markers of inflammation predict the long-term risk of developing chronic kidney disease: a population-based cohort study. Kidney Int. 2011;80:1231–8.

13. Mendoza JM, Isakova T, Cai X, Bayes LY, Faul C, Scialla JJ, et al. Inflammation and elevated levels of fibroblast growth factor 23 are independent risk factors for death in chronic kidney disease. Kidney Int. 2017;91:711–9.

14. Cottone S, Palermo A, Arseno R, Riccobene R, Guarner iM, Mulè G, et al. Relationship of fetuin-A with glomerular filtration rate and endothelial dysfunction in moderate-severe chronic kidney disease. JNEPHROL. 2010;23(1):62–9.
15. Maharem DA, Gomaa SH, El Ghandor MK, Mohamed EI, Matrawy KA, Zaytoun SS, et al. Association of serum fetuin-A and fetuin-A gene polymorphism in relation to mineral and bone disorders in patients with chronic kidney disease. The Egyptian Journal of Medical Human Genetics. 2013;14:337–52.

16. Mehrotra R. Emerging role for fetuin-A as contributor to morbidity and mortality in chronic kidney disease. Kidney Int. 2007;72:137–40.

17. Blaha V, Mistrik E, Dusilova-Sulkova S, Kalousova M, Andrys C, Blaha M, et al. Circulating fetuin-A predicts early mortality in chronic hemodialysis patients. Clin Biochem. 2009;42(10–11):996–1000.

18. Thakkinstian A, Chailurkit L, Warodomwichit D, Ratanachaiwong W, Yamwong S, Chanprasertyothin S, et al. Causal relationship between body mass index and fetuin-A level in the asian population: a bidirectional mendelian randomization study. Clin Endocrinol. 2014;81:197–203.

19. Guo V, Cao B, Cai C, Cheng K, Cheung B. Fetuin–A levels and risk of type 2 diabetes mellitus: a systematic review and meta–analysis. Acta Diabetol. 2010;55:87–98.

20. Ix J, Wassel C, Kanaya A, Vittinghoff E, Johnson K, Koster A, et al. Fetuin-A and Incident Diabetes Mellitus in Older Persons. JAMA. 2008;300(2):182–8.

21. Bunnag P, Ongphiphandhanakul B. Roles of fetuin-A in hypertension2016 June 20, 2018. Available from: http://www.thaiheart.org/images/column_1387023976/Fetuin_EGAT%2013Dec2013.pdf.

22. Guarneri M, Geraci C, Incalcaterra F, Arsena R, Mule G, Vaccaro F, et al. Subclinical atherosclerosis and fetuin-A plasma levels in essential hypertensive patients. HYPERTENS RES.2012:1–5.

23. Vathesatogkit P, Woodward M, Tanomsup S, Ratanachaiwong W, Vanavanan S, Yamwong S, et al. Cohort profile: the electricity generating authority of Thailand study. Int J Epidemiol. 2012;41(2):359–65.

24. National Kidney Foundation. CKD-EPI Creatinine Eq. 2009 [Available from: https://www.kidney.org/content/ckd-epi-creatinine-equation-2009.

25. Evidence-Based Medicine Consults. Lab Test: Uric Acid (Serum) Level [Available from: https://www.ebmconsult.com/articles/lab-test-uric-acid-level.

26. World Health Organization & International Diabetes Federation. Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia: report of a WHO/IDF consultation. World Health Organization 2006. Available from: https://apps.who.int/iris/handle/10665/43588.

27. Preacher K, Hayes A. Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. Behav Res Methods. 2008;40:879–91.

28. Texas StataCorp LP. Stata Release 15. Statistical software, College Station. 2018.

29. Ix J, Shlipak M, Sarnak M, Beck G, Greene T, Wang X, et al. Fetuin-A is not associated with mortality in chronic kidney disease. Kidney Int. 2007;72:1394–9.

30. Ketteler M, Bongartz P, Westenfeld R, Wildberger J, Mahnken A, Böhm R, et al. Association of low fetuin-A (AHSG) concentrations in serum with cardiovascular mortality in patients on dialysis: a cross-sectional study. Lancet. 2003;361:827–33.
31. Caglar K, Yilmaz MI, Saglam M, Cakir E, Acikel C, Eyleten T, et al. Short-Term Treatment with Sevelamer Increases Serum Fetuin-A Concentration and Improves Endothelial Dysfunction in Chronic Kidney Disease Stage 4 Patients. Clin J Am Soc Nephrol. 2008;3:61–8.

32. Yilmaz MI, Sonmez A, Saglam M, Yaman H, Kilic S, Demirkaya E, et al. FGF-23 and vascular dysfunction in patients with stage 3 and 4 chronic kidney disease. Kidney Int. 2010;78:679–85.

33. Hermans M, Brandenburg V, Ketteler M, Kooman J, van der Sande F, Boeschoten E, et al. Association of serum fetuin-A levels with mortality in dialysis patients. Kidney Int. 2007;72(2):202–7.

34. Lebreton JP, Joisel F, Raoult JP, Lannuzel B, Rogez JP, Humbert G. Serum concentration of human alpha2-HS glycoprotein during the inflammatory process: evidence that alpha2-HS glycoprotein is a negative acute-phase reactant. J Clin Invest. 1979;64:1118–29.

35. Altuntaş A, Yiğit A, Uz E, İnal S, Kidir V, Aydin B, et al. The relationship between serum fetuin a levels and fetuin gene polymorphism in hemodialysis patients. Biomed Res. 2017;28(2):495–502.

36. Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, et al. Obesity and Cardiovascular Disease: Pathophysiology, Evaluation, and Effect of Weight Loss: An Update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease From the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. Circulation. 2006;113:898–918.

37. Cohen E, Fraser A, Goldberg E, Milo G, Garty M, Krause I. Association between the body mass index and chronic kidney disease in men and women. A population-based study from Israel. Nephrol Dial Transplant. 2013;28(Suppl. 4):iv130–5.

38. Lyngdoh T, Vuistiner P, Marques-Vidal P, Rousson V, Waerber Gr, Vollenweider P, et al. Serum Uric Acid and Adiposity: Deciphering Causality Using a Bidirectional Mendelian Randomization Approach. PLoS ONE. 2012;7(6):e39321.

39. Ford E, Williamson D, Liu S. Weight change and diabetes incidence: findings from a national cohort of US adults. Am J Epidemiol. 1997;146:214–22.

40. Thawornchaisit P, De Looze F, Reid CM, Seubsman S, Sleigh A, Team TCS. Health-Risk Factors and the Prevalence of Hypertension: Cross-Sectional Findings from a National Cohort of 87 143 Thai Open University Students. Global Journal of Health Science. 2013;5(4):126–41.

41. Fox C, Matsushita K, Woodward M, Bilo HJ, Chalmers J, Heerspink HJL, et al. Associations of kidney disease measures with mortality and end-stage renal disease in individuals with and without diabetes: a meta-analysis. Lancet. 2012;380(9854):1662–73.

42. Mahmoodi B, Matsushita K, Woodward M, Blankestijn P, Cirillo M, Ohkubo T, et al. Associations of kidney disease measures with mortality and end-stage renal disease in individuals with and without hypertension: a meta-analysis. Lancet. 2012;380(9854):1649–61.

43. De Cosmo S, Viazzi F, Pacilli A, Giorda C, Ceriello A, Gentile S, et al. Predictors of chronic kidney disease in type 2 diabetes-A longitudinal study from the AMD Annals initiative. Medicine. 2016;95(27(e4007):1–7.

44. Bailey RA, Wang Y, Zhu V, Rupnow MF. Chronic kidney disease in US adults with type 2 diabetes: an updated national estimate of prevalence based on Kidney Disease: Improving Global Outcomes
(KDIGO) staging. BMC Res Notes. 2014;7(415):1–7.

45. Gambara V, Mecca G, Remuzzi G, Berfani T. Heterogeneous nature of renal lesions in type II diabetes. J Am Soc Nephrol. 1993;3:1458–66.

46. Mazzucco G, Bertani T, Fortunato M, Bernardi M, Leutner M, Boldorini R, et al. Different patterns of renal damage in type 2 diabetes mellitus: A multicentric study on 393 biopsies. Am J Kidney Dis. 2002;39(4):713–20.

47. nih.gov. alpha 2-HS glycoprotein, [AHSG, Homo sapiens (human)] [Available from: ].

48. Sun Z-L, Xie Q-Y, Guo G-L, Ma K, Huang Y-Y. Serum Fetuin-A Levels in Patients with Cardiovascular Disease - A Meta-Analysis. BioMed Research International. 2014:1–9.

49. Hussein N, Mahmoud O, Zahran M, Rafaat M. Serum Fetuin-A in Chronic Renal Disease Patients: Contribution to Endothelial Dysfunction and Hemostatic alteration. Journal of American Science. 2010;6(12):1098–105.

50. Zhou Z, Ji Y, Ju H, Chen H, Sun M. Circulating Fetuin-A and Risk of All-Cause Mortality in Patients With Chronic Kidney Disease: A Systematic Review and Meta-Analysis. Front Physiol. 2019;10(966):1–9.

51. Caglar K, Yilmaz M, Saglam M, Cakir E, Kilic S, Eyileten T, et al. Endothelial dysfunction and fetuin A levels before and after kidney transplantation. Transplantation. 2007;83(4):392–7.

52. Landmesser U, Hornig B, Drexler H. Endothelial function: a critical determinant in atherosclerosis? Circulation 2004;109(Supp 1):II27-II33.

53. Cersosimo E, DeFronzo RA. Insulin resistance and endothelial dysfunction: the road map to cardiovascular diseases. Diabetes Metab Res Rev. 2006;22:423–36.

54. Malyszko J. Mechanism of endothelial dysfunction in chronic kidney disease. Clin Chim Acta. 2010;411:1412–20.

Figures
Figure 1
Causal association pathways between fetuin-A and eGFR

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