***Method***

**Literature search strategy**

We adopted a systematic approach to identify articles focusing on VC in patients across all stages of CKD, using the following MeSH or Emtree keywords: ‘vascular calcification’, ‘male’ or ‘female’, and ‘renal insufficiency, chronic’ or ‘renal replacement therapy’, from databases including PubMed, MEDLINE, EMBASE, Google Scholar, and Cochrane Reports between 1968 and 06 May 2021. Inclusion criteria were original investigations involving human subjects, reporting data of the gender variable and any types of VC or its potential functional regulators, among our target population with various stages of CKD. Identified studies were initially screened by two investigators (P.Y.W. and C.T.C.) independently, followed by the exclusion of review articles, those without abstract available for discrimination purpose, those that did not compare the influence on or the prevalence/course/risk of VC or its related regulators between different genders, or those that focus on non-CKD target population (Figure 1). We subsequently reviewed the abstract of the post-screening articles and their reference lists, in order to uncover additional studies containing original data addressing similar topics. Discrepancies between the two reviewers regarding article eligibility was resolved by arbitration from a senior investigator (J.W.H.).

We extracted relevant parameters from the identified studies, including the year and authors of publication, the stages of participants’ CKD at baseline, methods for measuring VC status and severities, the distribution of gender and/or the gender as a predictor of VC or its regulators, results from univariate and multivariate analyses of clinical features between male and female participants. We organized results according to the following categories: gender-related difference in the prevalence, severities and/or courses of vascular calcification; certain gender as a risk factor for incident/worsening VC; and gender as a potential modifier for regulators of VC.

***Literature findings***

In mostly CKD (non-dialysis) was defined according to the estimated glomerular filtration rate according to the Modification of Diet in Renal Disease, but very few studies evaluated CKD based on elevated serum creatinine levels. Staging of CKD, whichever available, was performed based on the Kidney Disease Improving Global Outcome (KDIGO) criteria (1).

Factors adjusted for in the multivariate analyses included at least age and gender in all studies and could further include parameters such as hormones, microRNAs, proteins and laboratory profiles.

***Gender-related differences in the prevalence of CKD-associated VC***

A total of 101 articles were retrieved and summarized (2-102). Overall, 92 studies included patients with ESRD, of which 66 articles with patients on dialysis and 15 articles with patients at CKD stage 5T. Over 50 studies concluded a neutral effect of gender on the prevalence of vascular calcification. In another 44 articles male are more prevalent in patients with more severe vascular calcification, whereas there are only 5 studies in which female were more common in patients with severe vascular calcification. In articles without patients with ESRD, 8 studies concluded that male gender was more prevalent in severe calcification group, while 4 concluded neutral.

***Gender-related differences in the adjusted risk of CKD-associated VC***

A total of 66 articles analyzed the gender-associated risk of different sites of VC among patients with CKD (16, 18, 20, 23, 24, 34, 39-41, 43, 44, 60, 62, 64, 66, 69, 71, 76-78, 82, 88, 89, 92, 93, 95-130). 14 studies included only predialysis patients, of which male gender was a risk factor for CKD-associated VC in 9 studies (18, 20, 24, 34, 88, 92, 95, 100, 108, 115, 125). Of these studies, 43 articles reported that male gender predicts vascular calcification in patients with CKD, while 19 articles reported neutral effect and 2 implied female gender as a determinant.

***Potential mediators of gender-related differences in VC***

A total of 53 articles addressed potential modifiers of gender-VC relationship among patients with CKD (12, 16, 19, 34, 35, 37, 42, 62, 82, 83, 86, 91, 105, 106, 110, 128, 131-167).

Potential mediators which showed no gender-related differences in patients with or without VC are copeptin, ECF excess, epicardial fat, fetuin-A, CRP, free p-cresylglucuronide, magnesium, MGP Genotype T-138C, microRNA-125b, nutrition (albumin), serum irisin, serum pre-B cell colony-enhancing factor/visfatin, statin use, urinary calcium excretion, and VKORC1 (vitamin K epoxide reductase complex 1) polymorphism.

Those mediators which were higher in female were HDL, adiponectin, serum copper, tissue AGEs (surrogated by skin autofluorescence), total fat mass, and more Vitamin D deficiency. However, LDL was lower in female patients.

Male patients had more pericardial fat, sclerostin (mostly), serum ACE2, and signal peptide-CUB-EGF domain-containing protein 1 (SCUBE1).

Other mediators remain in controversies are Klotho (neutral\*1 / female higher\*1), Dp-uMGP (male lower), dp-cMGP (neutral), FGF-23 (neutral\*1 / female higher\*1), parathyroid hormones (neutral\*1 / female higher\*1), serum phosphorus (female higher\*1 / neutral\*1), OPG (male\*3 / female\*2 / neutral\*3).

***Discussion***

*Cardiovascular comorbidities in CKD and Gender differences*

The incidence of vascular calcification in patients with predialysis CKD is about 40%, and the incidence increases to over 70% as CKD progressed (124, 168). The retrieved previous studies showed that in patients with vascular calcification, the male gender serves as the main predictor for vascular calcification whether in patients with predialysis CKD, on dialysis, or in patients who had undergone kidney transplantation.

In 2018, based on the annual report published by the United States Renal Data System, the incidence for each cardiovascular disease was higher in male CKD patients (169). In male CKD patients, the incidence of any CVD, CAD, and PAD was 71.3%, 44.8%, and 29.3% respectively. However, in female CKD patients, the incidence of each kind of CVD is lower compared with male patients, in which any CVD, CAD, and PAD each accounts for 62.4%, 29.1%, and 24.0% (169).

In patient with calciphylaxis, a rare comorbidity of CKD which can cause calcification in various tissues, female gender is more prevalent, ranging from 60% to 100% (170-179).

Regarding the cardiovascular comorbidities-related procedures, the incidence of percutaneous coronary interventions among male and female CKD patients is 2.67% and 1.29%, respectively. As to coronary artery bypass surgery in patients with CKD, the incidence of the procedure in male patients outnumbered the incidence in female patients by threefold. Implantable cardioverter defibrillator/cardiac resynchronization therapy defibrillator in male and female CKD patients is 0.32% and 0.09%. The incidence of carotid artery stenting and carotid endarterectomy was 0.44% in male and 0.30% in female patients with CKD on the other hand (169).

*Phosphorus and Gender differences*

Phosphorus was long believed to act a significant effect on cardiovascular calcification in chronic kidney disease-mineral bone disease by its nature of binding and depositing with calcium. However, in this review we retrieved only two studies concerning the effect of gender on phosphorus in patients with chronic kidney disease. In a study conducted by Block *et al.,* female gender served as a determinant of higher phosphorus in patients undergoing hemodialysis (133). While another study showed that gender did not modify the level of serum phosphorus in patients with all stages of CKD (167). Female gender may serve as a determinant of vascular calcification through its effect on the level of serum phosphorus. Controlling serum phosphorus of patients undergoing hemodialysis is suggested.

*Parathyroid hormone, sclerostin, FGF-23 and Gender differences*

Parathyroid hormones had been shown to induce phosphaturic response, decrease reabsorption of phosphate from urine and increase uptake of calcium and phosphate from intestines and bone into the bloodstream. Whether gender, parathyroid hormone, and vascular calcification are correlated is an unresolved problem. González-Parra E *et al.* showed that female gender was correlated with higher level of parathyroid hormone in predialytic CKD patients (139). However, Jean *et al.* showed that gender and the level of parathyroid hormone was not related (144). (Patients with higher PTH showed increased risk of low bone mass, Kirkpantur *et al.* inferred a negative relation between serum PTH level and bone mineral densities, with a standard regression coefficient of -0.21– -0.33 (180). Intact PTH is shown to be related with Gensini vascular calcification score, with a correlation coefficient of 0.152, p = 0.044 (181)) In a Belgium cohort with 268 kidney transplant patients, lower PTH was identified as an independent determinant of higher serum sclerostin levels, which was related to lower baseline aortic calcification score (106). Moreover sclerostin was suggested to play a role in reducing mineralization during the late phase of vascular calcification in hemodialysis patients (163). Though evidence remain scarce, monitoring and controlling PTH levels through medication and lifestyle modification in CKD patients are still warranted.

Reduced clearance of phosphorus results in a higher level of FGF-23 secreted by osteocytes, contributing to secondary hyperparathyroidism through the negative effect of FGF-23 on calcitriol (182), whereas the way gender associated with vascular calcification through modifying FGF-23 was still under investigation. Turan *et al.* confirmed, although small, the risk for CACS per 50 pg/mL increase of FGF-23 in 224 hemodialysis patients (82). Tamei *et al.* conducted a study including 127 hemodialysis patients and inferred that FGF-23 serves as a significant modifier for aortic artery calcification score over progression 5 year of follow-up (127). A study conducted by González-Parra E *et al.* showed that female gender was related to higher FGF-23 levels (139). Nevertheless, Turan *et al.* concluded that gender was not associated with FGF-23 in regard to gender prevalence among different tertiles of FGF-23 (82).

Interestingly, patients with abdominal aortic calcification of Kauppila index > 5 are prone to have impaired FGF23-induced phosphaturic response, while the impairment of PTH-induced phosphaturia was not noted (24).

*Vitamin D deficiency and Gender differences*

Female gender had been shown to be associated with 25-hydroxyvitamin D (25D) deficiency in hemodialysis patients (12, 143). Whether gender determines vascular calcification through vitamin D deficiency remained unclear. Calcidiol deficiency had been described to be associated with reduced sun exposure, reduced skin synthesis, reduced ingestion of foods with vitamin D, loss of vitamin D binding protein with proteinuria (183). The relationship between the serum vitamin D level and vascular calcification scores in hemodialysis patients was of some controversy. Chang *et al.* illustrated a negative correlation of 25D levels with the Kauppila index in 289 hemodialysis patients from a cohort in South Korea (12). Wang *et al.* revealed a similar result, where 25D levels were negatively related to the Kauppila index in 126 hemodialysis patients from China (184). In both studies, 25D levels lost their significances after adjustment. The relationship between serum 25D levels and vascular calcification in subgroups of different genders remained unclear, but the evidence above could support the association between female gender and vitamin D deficiency.

*Osteoprotegerin and Gender differences*

A study conducted by Scialla *et al.* confirmed a 30% increase in the ratio of aortic pulse wave velocity (PWV) predicted by higher osteoprotegerin when unadjusted had been elucidated. However, the effect size was lower (ratio = 1.10) when adjusted for traditional/non-traditional risk factors, and cortical bone mineral content measured by peripheral quantitative computed tomography of the left tibia (156). Male gender had been widely concerned as a risk factor for higher vascular calcification scores throughout the studies included in this review. Interestingly, female gender may still play a role in vascular calcification in CKD patients. Osteoprotegerin had been shown to predict CACS ≥ 100 with a cutoff value of 757.7 pg/mL alongside with male gender (OR 4.95, 95% CI 2.36–10.37) in a French cohort with 133 patients with CKD stages 1 to 5 (64). Although female gender was associated to a 10.2% higher serum osteoprotegerin, the association was not adjusted, indicating a lower evidence (156).

*Fetuin-A and Gender differences*

Fetuin-A had been shown to be negatively related to bone mineral density, with standard regression coefficients ranging between -0.29 and -0.41 at different sites (radial, femur neck, and femur trochanter) (180). Interestingly, the Gensini score assessing the extent of coronary artery disease significantly correlated in univariate analysis with higher fetuin-A levels (R = 0.491; P = 0.001) (181). Moreover, low fetuin-A levels were risk factors for all-cause mortality in hemodialysis patients (HR 2.3, 95% CI 1.2–4.5) (149).

**Supplementation managements for vascular calcification in chronic kidney disease**

Vitamin D3… Vitamin K supplements and antagonist … Warfarin-treated male patients had more vertebral fractures (77.8 vs. 57.7%, p<0.04), but not females (42.1% vs. 48.4%, p=0.6). vitamin K deficiency… Omega-3 fatty acid supplementation…

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