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Estradiol and mortality in women with end-stage kidney disease

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ABSTRACT

Background. Young women with end-stage kidney disease (ESKD) have early menopause compared with women in the general population and the highest mortality among the dialysis population. We hypothesized that low estrogen status was associated with death in women with ESKD.

Methods. We measured estradiol and sex hormone levels in female ESKD patients initiating hemodialysis from 2005 to 2012 in four Canadian centers. We divided women into quintiles based on estradiol levels and tested for associations between the estradiol level and cardiovascular (CV), non-CV and all-cause mortality. Participants were further dichotomized by age.

Results. A total of 482 women (60 ± 15 years of age, 53% diabetic, estradiol 116 ± 161 pmol/L) were followed for a mean of 2.9 years, with 237 deaths (31% CV). Estradiol levels were as follows (mean ± standard deviation): Quintile 1: 19.3 ± 0.92 pmol/L; Quintile 2: 34.6 ± 6.6 pmol/L; Quintile 3: 63.8 ± 10.6 pmol/L; Quintile 4: 108.9 ± 19.3; Quintile 5: 355 ± 233 pmol/L. Compared with Quintile 1, women in Quintiles 4 and 5 had significantly higher adjusted all-cause mortality {hazard ratio [HR] 2.12 [95% confidence interval (CI) 1.38–3.25] and 1.92 [1.19–3.10], respectively}. Similarly, compared with Quintile 1, women in Quintile 5 had higher non-CV mortality [HR 2.16 (95% CI 1.18–3.96)]. No associations were observed between estradiol levels and CV mortality. When stratified by age, higher quintiles were associated with greater all-cause mortality (P for trend <0.001) and non-CV mortality (P for trend = 0.02), but not CV mortality in older women.

Conclusions. In women with ESKD treated with hemodialysis, higher estradiol levels were associated with greater all-cause and non-CV mortality. Further studies are required to determine the mechanism for the observed increased risk.

Keywords: cardiovascular, end-stage renal disease, mortality, estradiol, women

INTRODUCTION

Women with end-stage kidney disease (ESKD) have an abnormal sex hormone profile characterized by hypothalamic pituitary disturbances and low estradiol levels [1–3]. In contrast the general population, young women with ESKD are at the highest risk of mortality compared with age-matched men with ESKD [4]. The reasons for this high risk of death are unclear, although decreased lifetime exposure to estrogen has been postulated to contribute to increased mortality [5–7]. In the general population, menopause (and particularly early menopause) is associated with an increase in the incidence of cardiovascular (CV) disease and mortality [6–11]. The mean age of menopause in the female dialysis population is 47 years [12], which is 5 years earlier than the general population [13]. Premenopausal women with coronary artery disease are more likely to have hypothalamic hypoestrogenemia [14], a condition that, in primates, is related to premenopausal atherosclerosis [15–17].

Given the association between ESKD and hypothalamic pituitary abnormalities that lead to hypoestrogenemia, we sought to determine the relationship between estradiol levels and all-cause, CV and non-CV mortality in a prospective cohort of women with ESKD initiating hemodialysis. We hypothesized that low estradiol levels would be associated with increased mortality and specifically CV mortality.

MATERIALS AND METHODS

Study design

This was a prospective multicenter cohort study using data from the Canadian Kidney Disease Cohort Study (CKDCS), the methodology of which is described elsewhere [18]. Briefly, patients initiating conventional hemodialysis in four dialysis centers in Canada (located in Calgary, Edmonton, Ottawa and Vancouver) from February 2005 to November 2012 were enrolled and prospectively followed. A principal investigator was responsible for recruitment, data collection and follow-up at each site. Information

KEY LEARNING POINTS

What is already known?

- Women with end-stage kidney disease (ESKD) have an abnormal sex hormone profile characterized by hypothalamic pituitary disturbances and low estradiol levels.
- In contrast with the general population, young women with ESKD are at the highest risk of mortality compared with age-matched men with ESKD.
- The reasons for this high risk of death are unclear, although decreased lifetime exposure to estrogen has been postulated to contribute to increased mortality.

What this study adds?

- In this multicenter prospective cohort study of women with ESKD initiating hemodialysis, we aimed to examine the association between estradiol levels and mortality.
- In contrast to our hypothesis, we observed that, overall, women with higher levels of estradiol had a significantly higher risk of all-cause and non-CV mortality, which appeared to be mainly driven by women with diabetes.
- However, when stratified by age, higher estradiol levels were associated with higher adjusted all-cause and CV mortality only in older women.

What impact this may have on practice or policy?

- Given the high mortality in this population, investigations into novel and sex-specific risk factors are of the utmost importance.
- Further studies are required to determine the mechanism by which increased estradiol may increase the risk of death in the ESKD population or whether an intervention to reduce estradiol levels would result in decreased mortality in this population.
- We believe the strong association observed in this study merits attention.

on patient demographics, medical history and mortality was collected. Ethics approval for the study was obtained from the institutional review boards of all participating centers and was conducted according to the Declaration of Helsinki [18].

Study participants

All incident female ESKD CKDCS participants ≥18 years of age were eligible (Figure 1).

Data collection

Consenting participants underwent a structured interview at baseline to collect information on demographic characteristics and medical history. Information from the patients' clinical record was used to supplement the medical history. Follow-up visits were conducted at 6 months and then annually for 5 years. After the fifth year, follow-up visits were conducted every 5 years.

Sera from blood samples were collected within 3 months of initiation of hemodialysis and were processed and frozen in 0.5 mL cryovials at −85°C within 72 h of sample collection. There was no attempt to collect specimens based on the menstrual cycle due to previous studies indicating the common prevalence of amenorrhea in the ESKD population [19]. The frozen sera samples were analyzed for serum estradiol, follicle-stimulating hormone (FSH) and prolactin levels at a central laboratory.

Outcome measures

The primary outcome was all-cause mortality, with secondary outcomes of CV and non-CV mortality. The date of death of study participants was identified through regular follow-up by study coordinators. The cause of death was independently adjudicated by two physicians using information from medical records, hospital discharge summaries and autopsy reports (if available); any disagreement was resolved by consensus. If independent assessment of cause of death was not available, then data were obtained from Canadian Vital Statistics.

Deaths due to acute myocardial infarction, atherosclerotic CV disease, circulatory complications of diabetes, ischemic heart disease, hypertrophic cardiomyopathy, stroke, aneurysm, vascular disorder, sudden cardiac death and peripheral vascular disease were classified as CV. All other deaths were classified as non-CV.

Statistical analysis

Baseline characteristics are presented as mean ± standard deviation (SD) or proportions unless otherwise stated. Participants were stratified into quintiles based on serum estradiol levels, as menopause status was unavailable for patients. Cox proportional hazards models were used to determine the association between categories of estradiol and each of the outcomes of all-cause, CV and non-CV mortality. Participants were censored at switching of modality, emigration to another province, loss to follow-up or end of study (31 March 2016). Participants in the group with the lowest level of estradiol were used as the referent group for all analyses. Additionally, to determine the association between age, estradiol levels and mortality, participants were stratified as ≥51 and <51 years, the average age of menopause in the general population [13]. All Cox regression models were adjusted for potential confounders including age, body mass index (BMI), diabetes, smoking status, hypertension, history of coronary artery disease, glomerulonephritis and prolactin levels [20]. Additionally, interaction by age was assessed in all Cox regression models using age as both a categorical (≥51 and <51 years) and a continuous variable. A two-sided P-value <0.05 was considered statistically significant for all analyses.

In sensitivity analyses, we explored differences in adjudicated deaths versus cause of deaths defined by vital statistics data, as well as the potential effect of duration of storage and

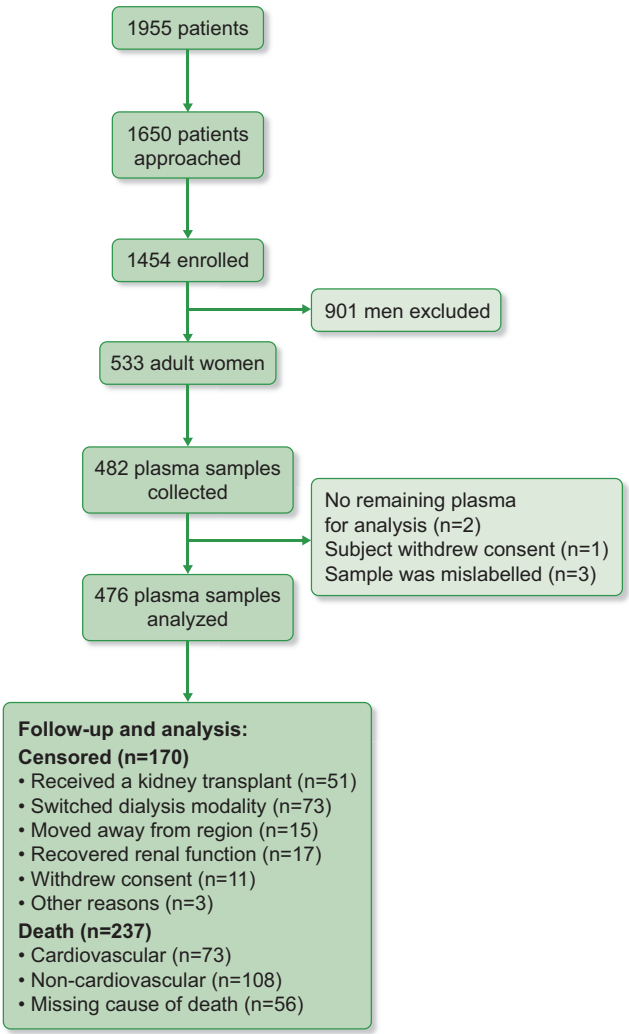

FIGURE 1: Creation of the cohort.

hemodialysis center on serum estradiol measurements by plotting estradiol levels versus time since collection. Limited studies have suggested the median age of menopause is 48 years in the population with ESKD [12] and analyses were repeated using this age as a cut-off, as well as by diabetic status.

As we did not have information regarding menstrual status, the Women's Ischemia Syndrome Evaluation (WISE) algorithm was used to determine the menopausal status of women with ESKD [21]. Briefly, women were classified as follows: premenopausal if (i) FSH <10 IU/L or (ii) FSH 10–20 IU/L and estradiol >184 pmol/L; perimenopausal if (i) FSH 10–20 IU/L and estradiol <184 pmol/L, (ii) FSH 20–30 IU/L, (iii) FSH >30 IU/L and estradiol ≥184 pmol/L or (iv) age ≥45 years and estradiol ≥734.2 pmol/L; and postmenopausal if FSH >30 IU/L and estradiol <184 pmol/L. Women were also stratified based on estradiol levels above and below the median and FSH levels above and below the median to characterize their sex hormone profiles. All analyses were performed using Stata (version 12; StataCorp, College Station, TX, USA).

RESULTS

Study participants

Of 1955 patients with ESKD initiating hemodialysis, 1650 were approached and ultimately 476 were included in final analysis (see Figure 1 for details).

Baseline characteristics

The baseline characteristics of the 476 women are listed in Table 1. The mean age of participants was 60 ± 15 years and the majority were Caucasian (75%). Most of the women had known hypertension (87%) and 53% had diabetes. The participants were followed for an average of 2.9 ± 2.5 years {median 2.45 [interquartile range (IQR) 3.5]}.

The baseline characteristics of participants stratified by estradiol quintile are shown in Table 1. Age was significantly different between the quintiles (P for trend <0.0001) and women in the lower estradiol quintiles had longer follow-up compared with women in the higher quintiles (P = 0.01).

Sex hormone levels

The mean serum estradiol level for the cohort was 116 ± 162 pmol/L [median 64 pmol/L (IQR 94)], the mean serum FSH level was 58 ± 55 IU/L [median 49 (IQR 89)] and the mean serum prolactin level was 68 ± 137 IU/L [median 27 (IQR 31)] (Table 1).

Sex hormone levels stratified by estradiol level are presented in Table 1. As expected, women in the lowest quintiles had higher FSH levels compared with women in the higher quintiles (P < 0.0001). Serum prolactin levels were not significantly different across groups (P = 0.3).

Mortality in women with ESKD

A total of 237 participants died over a mean follow-up of 2.9 years and the incidence rate of 16.89 deaths per 100 person-years (Table 2). Seventy-three (31%) of the deaths were due to CV causes and the incidence rate was 5.2 deaths per 100 person-years (Table 2). A toal of 108 (46%) deaths were due to non-CV causes and the incidence rate was 7.7 deaths per 100 person-years (Table 2). Of the 237 deaths, the cause of death in 165 (69%) was adjudicated by independent reviewers. For 16 of the 165 deaths the cause of death was determined through vital statistics data. The cause of death was missing for 56 (23%) of the participants.

Associations between estradiol and all-cause mortality

Compared with the reference group (lowest quintile of serum estradiol levels), the two groups with the highest levels of serum estradiol had significantly greater all-cause mortality after adjustment for covariates {hazard ratio [HR] 2.17 [95% confidence interval (CI) 1.41–3.32] and HR 1.92 [95% CI 1.19–3.10], respectively} (Table 3 and Figure 2). There was no significant interaction for age (<51 versus ≥51 years; P = 0.055). Upon stratification based on age, women ≥51 years showed higher all-cause mortality in the two highest estradiol level groups [HR 2.37 (95% CI 1.50–3.76) and HR 2.24 (95% CI

1.30–3.89); P for trend <0.001] (Table 3), however, this association was not observed in women <51 years of age (Table 3).

Associations between estradiol level and CV mortality

No association between estradiol levels and CV mortality was observed across the groups after adjusting for covariates (Table 3). There was significant interaction for age (<51 versus ≥51 years; P = 0.02). Upon stratification based on age and after adjustment, women ≥51 years of age showed a significant association with higher CV mortality in the group with highest estradiol levels [HR 2.93 (95% CI 1.13–7.55); P = 0.04] (Table 3). However, this association was not observed in women <51 years of age (Table 3).

Associations between estradiol level and non-CV mortality

The quintile with the second highest level of serum estradiol had significantly greater non-CV mortality compared with the referent group after adjustment for covariates [HR 2.20 (95% CI 1.30–4.03)] (Table 3). The interaction for age was not significant (<51 versus ≥51 years; P = 0.40).

Sensitivity analysis

Exclusion of nonadjudicated causes of death did not significantly alter the results. No association was found between estradiol levels and date of serum collection (P = 0.2). There was no observed difference according to center. Further analyses using 48 years as a cut-off for age did not significantly alter the results. When stratified by diabetes status, higher estradiol levels were associated with significantly greater all-cause (P for trend = 0.001) and non-CV mortality (P for trend = 0.04), but not CV risk (P = 0.06), in women with diabetes after adjustment for confounders. No associations were observed in women without diabetes (all-cause mortality, P = 0.18; CV mortality, P = 0.6; non-CV mortality, P = 0.83).

Menopausal status using WISE classification

Using the WISE classification, 35% ($n = 164$) were classified as premenopausal, 7% ($n = 34$) were classified as perimenopausal and 58% ($n = 278$) were classified as postmenopausal. The mean age of the women in each group was 50 ± 17 years for premenopausal women, 64 ± 14 years for perimenopausal and 66 ± 11 years for postmenopausal women.

DISCUSSION

In this multicenter prospective cohort study of women with ESKD initiating hemodialysis, we aimed to examine the association between estradiol levels and mortality. In contrast to our hypothesis, we observed that, overall, women with higher levels of estradiol had a significantly higher risk of all-cause and non-CV mortality, which appeared to be mainly driven by women with diabetes. However, when stratified by age, higher estradiol levels were associated with higher adjusted all-cause and CV mortality only in older women.

To our knowledge, this is the first prospective study examining the associations between estradiol levels and mortality in women with CKD. Our study has some similarities to a previous retrospective study examining the relationship between serum estradiol and mortality in 283 prevalent women with ESKD treated with hemodialysis [5]. Over a mean of 3 years of follow-up a U-shaped association was observed, with the high and low tertiles of estradiol being associated with greater all-cause and CV mortality, while we observed a positive association between estradiol level and mortality. These differences could be due to several reasons. First, it could be attributed to different age cut-offs in the two populations. Our study included women with a wider age range and we stratified our results based on both the mean age of menopause in the general North American population [13] as well as the mean and median age of menopause of the dialysis population [12]; in comparison, Tanrisev *et al.* [5] included only women >45 years of age. Second, women ≥51 years of age in our study had lower mean estradiol levels as compared with the cohort of women in the study by Tanrisev *et al.* [5] (78 ± 5 versus 105 ± 57 pmol/L, respectively); however, the estradiol levels of older women in our study were similar to those previously published [12]. Finally, our study included incident dialysis patients, and serum estradiol levels were measured within the first 3 months of dialysis initiation. In comparison, the women included in the study by Tanrisev *et al.* [5] were on dialysis for at least 6 months.

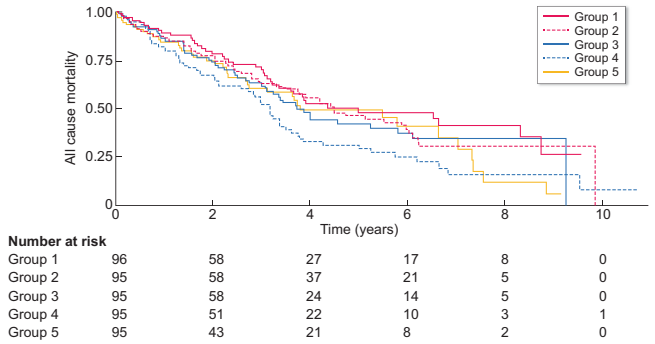

FIGURE 2: Risk of mortality in association with estradiol level.

Previous studies have suggested that earlier menopause, even in the absence of surgical removal of ovaries or chemotoxicity, is associated with decreased survival [22, 23], prompting international medical associations to issue guidelines suggesting the use of postmenopausal hormone therapy until at least the age of natural menopause in women with primary ovarian insufficiency [24–27], although there is uncertainty as to how or if these guidelines apply to the population with kidney disease [28]. Of note, while estradiol is purported to be the sex hormone responsible for CV protection [29], a lack of association between estradiol levels (independent of postmenopausal hormone use) and CV outcomes has been reported [30], suggesting that other factors may be involved, and thus our observation of a positive association between estradiol levels and mortality is in keeping with the published literature. In our study, estradiol levels were associated with increased non-CV, but not CV, mortality. Younger women with ESKD on dialysis have a high risk of mortality, mainly due to non-CV causes such as malignancy and infection [4]. Sex hormones modulate the immune system, and a recent cross-species meta-analysis [31] suggested that endogenous estrogen suppresses cell-mediated immune function,

Table 1. Baseline characteristics, by estradiol quintile

	All	Estradiol <24 pmol/L	Estradiol 24–47 pmol/L	Estradiol 48–82 pmol/L	Estradiol 83–157 pmol/L	Estradiol >157 pmol/L	P-value (analysis of variance)
n	476	96	95	95	95	95	
Age (years), mean ± SD	60±15	64 ±11	65±13	63±15	60± 16	50± 16	<0.0001
BMI (kg/m^2), mean ± SD	27.1±8.6	26.8±6.8	26.8±7.2	28.2±8.4	26.8± 8.5	27.0± 11.6	0.9
SBP (mmHg), mean ± SD	142± 27	143±27	146±27	42.1±25.3	144± 28	136± 26	0.08
DBP (mmHg), mean ± SD	75± 15	74±14	75±14	73±14	74± 17	77± 16	0.3
Diabetes, %	53	54	48	50	58	47	0.8
Hypertension, %	87	84	95	89	87	83	0.51
CAD, %	28	22	33	32	29	23	0.9
Access, *n*							0.94
Fistula or graft	22	23	21	20	20	24	
Central venous catheter	78	77	79	80	80	76	
Etiology of ESKD, %							0.01
Glomerulonephritis	16	9	19	14	15	21	
Diabetes	42	37.5	49.5	50.5	41.1	31.6	
HTN	5	8.3	4.2	1.1	3.2	6.3	
PKD	6	4.2	3.2	3.2	4.2	13.7	
Other	31	41	24	31	37	27	
Follow-up (years), mean ± SD	2.9± 2.5	3.1±2.6	3.4±2.6	3.1±2.4	2.7±2.3	2.4±2.3	0.01
Smoking status, %							
Never	49	52	49	44	47	52	0.8
Current	15	10	15	17	13	21	0.1
Past	36	38	36	39	39	26	0.2
Estradiol (pmol/L)[a], mean ± SD	116± 161	19.3± 0.9	34.6± 6.6	63.8± 10.6	108.9± 19.3	355.0± 233	<0.0001
FSH (IU/L), mean ± SD	58.0± 55.0	88.7 ± 57.3	78.6± 53.9	58.2± 49.4	51.2± 50.6	13.6± 25.2	<0.0001
Prolactin (μg/L), mean ± SD	68.7± 137.2	90.3± 221.0	63.1± 101.7	53.8± 84.2	64.2± 107.3	68.1± 123.4	0.3

[a]Estradiol reference ranges: follicular phase, 90–700 pmol/L; luteal phase, 150–950 pmol/L; mid-cycle phase, 250–1500 pmol/L; 61–150 years or post-menopause, 0–150 pmol/L.
CAD: coronary artery disease; DBP: diastolic blood pressure; HTN: hypertension; PKD: polycystic kidney disease; SBP: systolic blood pressure.

Table 2. Unadjusted all-cause mortality, CV mortality and non-CV mortality by estradiol quintile

All women (*N* = 476)	All-cause mortality	CV mortality	Non-CV mortality
Events, *n* (%)	237 (49.8)	73 (15.3)	108 (22.7)
Incidence rate per 100 person-years (95% CI)	16.89 (14.87–19.18)	5.2 (4.14–6.54)	7.7 (6.37–9.29)
Group 1: estradiol <24 pmol/L (referent) (*n* = 96)			
Events, *n* (%)	40 (16.9)	12 (16.4)	19 (17.59)
Incidence rate per 100 person-years (95% CI)	13.14 (9.64–17.91)	3.94 (2.24–6.94)	6.24 (3.98–9.78)
Group 2: estradiol 24–47 pmol/L (*n* = 95)			
Events, *n* (%)	50 (21.1)	16 (21.92)	22 (20.37)
Incidence rate per 100 person-years (95% CI)	15.59 (11.81–20.57)	4.99 (3.06–8.14)	6.86 (4.52–10.42)
Group 3: estradiol 48–82 pmol/L (*n* = 95)			
Events, *n* (%)	47 (19.8)	13 (17.81)	23 (21.30)
Incidence rate per 100 person-years (95% CI)	16.15 (12.13–21.50)	4.47 (2.59–7.69)	7.9 (5.25–11.89)
Group 4: estradiol 83–157 pmol/L (*n* = 95)			
Events, *n* (%)	60 (25.3)	16 (21.92)	30 (27.78)
Incidence rate per 100 person-years (95% CI)	23.19 (18.00–29.86)	6.18 (3.79–10.10)	11.59 (8.11–16.58)
Group 5: estradiol >157 pmol/L (*n* = 95)			
Events, *n* (%)	40 (16.9)	16 (21.92)	14 (12.96)
Incidence rate per 100 person-years (95% CI)	17.52 (12.85–23.89)	7.01 (4.29–11.44)	6.13 (3.63–10.36)

which may explain the increased risk of non-CV death in women with higher estradiol levels.

The fact that the positive association between estradiol level and both all-cause and non-CV mortality was most pronounced in women with diabetes deserves mention. Diabetes alters the production of sex hormones [32]; conversely, sex hormone levels may influence diabetes risk and glycemic control [32], although a recent prospective study suggested that estrogens did not predict diabetes risk in women [33]. Estradiol has been reported to be lower [34], higher [35, 36] or similar [37] in women with diabetes compared with age-matched, nondiabetic female controls, although renal function was not reported in these studies. As diabetes is associated with a greater risk of death in both the kidney and non-kidney disease populations, the increased absolute risk of death in women with diabetes compared with women without diabetes observed in our study is not unexpected. It is possible that had the follow-up of our study been longer, a similar association between estradiol levels and mortality may have been observed in the nondiabetic women, although this remains speculative.

Table 3. Adjusted risk of all-cause mortality, CV mortality and non-CV mortality by age and estradiol quintile

Outcome	Estradiol Group 1	Estradiol Group 2, HR (95% CI)	Estradiol Group 3, HR (95% CI)	Estradiol Group 4, HR (95% CI)	Estradiol Group 5, HR (95% CI)	P for trend
All-cause mortality						
All ($n = 237$)	1 (referent)	1.31 (0.84–2.03)	1.35 (0.86–2.12)	2.16 (1.41–3.31)	1.86 (1.14–3.01)	<0.001
<51 years ($n = 39$)		0.30 (0.07–1.40)	0.50 (0.12–2.12)	0.53 (0.15–1.88)	0.48 (0.14–1.65)	
≥51 years ($n = 198$)		1.35 (0.86–2.16)	1.38 (0.85–2.23)	2.37 (1.50–3.74)	2.17 (1.25–3.64)	
CV mortality						
All ($n = 73$)	1 (referent)	1.30 (0.60–2.83)	1.07 (0.47–2.44)	1.48 (0.67–3.28)	2.02 (0.90–4.54)	0.10
<51 years[a] ($n = 16$)		0.20 (0.02–2.40)	1.29 (0.17–9.77)	0.34 (0.06–1.87)	0.53 (0.11–2.65)	
≥51 years[b] ($n = 57$)		1.62 (0.68–3.87)	1.10 (0.42–2.90)	1.82 (0.72–4.57)	2.96 (1.14–7.65)	
Non-CV mortality						
All ($n = 108$)	1 (referent)	1.23 (0.65–2.34)	1.40 (0.74–2.64)	2.19 (1.20–4.01)	1.23 (0.58–2.63)	0.07
<51 years[c] ($n = 14$)		0.31 (0.18–5.21)	0.64 (0.43–9.33)	0.63 (0.06–7.04)	0.42 (0.04–4.83)	
≥51 years[d] ($n = 94$)		1.21 (0.62–2.37)	1.32 (0.68–2.59)	2.41 (1.28–4.54)	1.43 (0.6–3.43)	

[a]$n = 3, 1, 3, 4, 5$ for each estradiol group, respectively.
[b]$n = 9, 15, 10, 12, 11$ for each estradiol group, respectively.
[c]$n = 1, 2, 3, 3, 5$ for each estradiol group, respectively.
[d]$n = 18, 20, 20, 27, 9$ for each estradiol group, respectively.

Our study also highlights the challenges of evaluating menopausal status in the population with kidney disease. Menopause is defined as the secondary absence of menses for at least 12 months [38]. Although women with kidney disease often stop menstruating, more frequent hemodialysis or kidney transplantation result in a resumption of menses, and thus the 'menopause' of kidney disease is a reversible state. In the healthy population, menstrual cycle variation results in differing estradiol levels [39]; however, just over a third of premenopausal-aged women with Stage 5d treated with dialysis actually have menses [1] and there is a lack of variation in estradiol levels during the menstrual cycle in young women treated with dialysis [40]. Given the unpredictability of menopausal status in the ESKD population and the known association between estradiol levels and CV risks, our primary hypotheses were based on serum estradiol levels as opposed to menopausal status.

As we did not have access to the menstrual status of the participants in our study, we used the WISE classification to determine menopausal status, a classification that uses hormonal levels and not menses. However, the biologic implausibility of results such as women >80 years of age being classified as premenopausal precluded us from using this classification for our analyses, and we conclude that the WISE classification is not valid in the population with ESKD.

While it is accepted that kidney disease is associated with earlier onset of amenorrhea, there is limited knowledge about the hormonal milieu in the setting of CKD [12, 19] and thus the potential role of sex hormones in mediating increased risk in women on dialysis is unclear; however, there could be several possible reasons for the observed results. Although the mean estradiol levels were within the normal range for women with ESKD in our study, the distribution of estradiol levels was narrower than what is observed in the general population [21, 41, 42]. Previous studies have shown decreased variability of estradiol levels in young women on hemodialysis compared with premenopausal women in the general population [43]. Furthermore, women in quintiles with higher estradiol levels also initiated dialysis at a younger age. As such, one potential reason for the increase in mortality risk in women with higher estradiol levels on dialysis may be an early loss of cyclic estradiol exposure. There is evidence to suggest the premature loss of estradiol exposure, through early menopause, in the general population is associated with higher overall mortality. In a cohort of 12 134 healthy women, later menopause was associated with a 2% decrease in risk per year delay in menopause onset [22]. Additionally, surgical menopause in women <45 years of age is associated with a 67% increase in mortality [23], although whether this increase in risk is associated with a loss of cyclic variance in estradiol levels is not known. Finally, contrary to what is observed in the general population, studies have shown a reverse association between traditional markers of CV risk, such as BMI and total cholesterol [44, 45], and mortality in patients with ESKD. It is possible that the relationship between estradiol levels and mortality follows a similar pattern in the ESKD population.

This study has strengths and limitations. First, a one-time measurement of estradiol was used; however, single measurements have been shown to be reliable measures of long-term sex hormone levels in previous studies [46, 47]. Second, data on hormone therapy use in this cohort were not available and therefore we cannot differentiate between associations of endogenous and exogenous estradiol and mortality. However, a previous study showed markedly low prescription rates of postmenopausal hormone therapy in the ESKD population, even prior to the Women's Health Initiative trials [48–50]. Third, due to the nature of cohort studies, residual confounding is always possible; however, we included several known risk factors for cessation of menses and mortality in the multivariate models. Finally, a previous study of women without kidney disease did not show any association between estradiol levels and CV risk [30]. However, menopause in the ESKD population is reversible with more frequent dialysis or kidney transplant, highlighting that the potential relationship between estradiol and mortality may be specific to the population with kidney disease. Given the high mortality in this population, investigations

into novel and sex-specific risk factors are of the utmost importance. While further studies are required to determine the mechanism by which increased estradiol may increase the risk of death in the ESKD population, or whether an intervention to reduce estradiol levels would result in decreased mortality in this population, we believe the strong association observed in this study merits attention.

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CONFLICT OF INTEREST STATEMENT

The authors report no conflicts of interest and funding sources had no role in study design, conduct, or reporting.

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