The utility of anti-Müllerian hormone in women with chronic kidney disease, on haemodialysis and after kidney transplantation

Short title: Anti-Müllerian hormone in women with renal failure

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Key Message

Anti-Müllerian hormone (AMH) is lower in young women with renal failure compared with age-matched healthy controls but not in those on haemodialysis. AMH decreases with age in women with renal failure in a similar manner to the general population. AMH may have a role as a marker of ovarian health in non-dialysis renal patients pursuing pregnancy.

Abstract

Women with renal disease have menstrual and gonadal dysfunction manifesting as hormonal imbalance. Anti-Müllerian hormone (AMH) has been described as a potential measure of the ovarian reserve. We examined circulating AMH levels in young women with renal failure divided into 3 groups, determined associations with clinical characteristics, and compared AMH with age-matched healthy individuals. AMH concentrations were measured in 77 women (mean age 32.9±5.4 years); 26 had chronic kidney disease (CKD) stages 3-5, 26 were on haemodialysis (HD), and 25 had a kidney transplant. Random AMH levels are highest in women on HD [HD 2.9 (1.1-5.2), CKD 1.6 (0.7-2.2), transplant 1.5 (1.0-4.2)ng/mL]. On multiple linear regression, AMH was 53% higher (95% CI 0.20-0.98, p=0.002) in women on HD and decreased by 20% per 5-year increase in age (p<0.001). AMH was 43% lower in women with renal failure compared with 600 age-matched controls [1.7 (0.9-3.8) vs 3.0 (1.9-5.0)ng/mL, p<0.001]; however, we found no difference in AMH between those on HD and healthy individuals [2.9 (1.1-5.2) vs 3.0 (1.9-5.0)ng/mL]. AMH may be a useful biomarker in female renal patients with non-dialysis dependent renal disease pursuing pregnancy. In contrast, AMH levels are higher in HD but unlikely to reflect ovarian reserve.

Keywords: Anti-Müllerian hormone; chronic kidney disease; haemodialysis; kidney transplant; fertility.

Introduction

Anti-Müllerian hormone (AMH) is a glycoprotein with a fundamental role in male sex differentiation. In women, AMH plays a critical role in folliculogenesis, with circulating levels directly reflecting the number of developing preantral follicles and indirectly the number of primordial follicles in the ovaries (Iliodromiti et al., 2015). As such, AMH is now recognised as the best available biomarker of both the functional and true ovarian reserve (Dewailly et al., 2014). Accurate quantitative assessment of the ovarian reserve by AMH (Anderson et al., 2015) has enabled prediction of reproductive lifespan, tailoring of fertility preservation and optimisation of assisted conception outcomes (Dewailly et al., 2014; Nyboe Andersen et al., 2016). The recent development of a fully automated Elecsys® AMH immunoassay (Gassner and Jung 2014) with enhanced sensitivity, specificity and reproducibility, has widened its clinical utility and enabled assessment of women with limited ovarian function.

Women with advanced chronic kidney disease (CKD) often have disturbances in the menstrual cycle and amenorrhea is common by the time the patient reaches end stage renal disease (ESRD) (Zingraff et al., 1982). The menstrual cycle typically remains irregular even after the initiation of maintenance dialysis. Consistent with this, pregnancy is extremely uncommon as one progresses from CKD stage 3 to dialysis (Zingraff et al., 1982). Conversely, fertility is frequently restored within a few months after successful kidney transplantation (Levidiotis et al., 2009). To date we are only aware of a single small study (n=60) assessing AMH in patients with renal failure(Sikora-Grabka et al., 2016). This study utilised a manual AMH ELISA, which was limited by complement interference, irreproducible results and limited sensitivity (Iliodromiti et al., 2015).

The aim of this study was to measure serum AMH concentrations in predialysis, dialysis and kidney transplant women of childbearing age; explore potential factors affecting AMH and compare AMH levels with age-matched healthy controls.

Materials and methods

Design and Participants

This was a single-centre cohort study of all women aged 18-40 years attending renal services between August 1, 2015 and March 31, 2016 in our catchment area (serving a population of approximately 1.5 million). Potential participants were identified from the electronic patient record used in our centre and by screening clinic lists. A letter was sent to all eligible patients to make them aware of the research, which included an opt in or opt out reply slip where they could suggest a way for the research staff to contact them to discuss the study further. If they opted in, they were contacted by a member of the research team to discuss the details of the study and organise a study visit. The protocol of the study was approved by the Research Ethics Committee (REC reference: 15/NS/0040) on 20th May 2015. The study was conducted in accordance with the Declaration of Helsinki, and written informed consent was obtained from all participants.

We measured serum AMH levels in three distinct groups of patients; women with CKD stages 3-5, women on haemodialysis (HD), and kidney transplant recipients. We excluded individuals with active or previous cancer (breast, ovarian, lymphoma, pelvic radiotherapy), ovarian surgery, current use of alkylating agent-based protocols, severe active illness and patients with inability to provide informed consent.

AMH levels from a multicentre study using the same assay in 600 age-matched healthy women with regular menstrual cycles, not on contraception were used as the reference group (Anckaert et al., 2016).

Baseline data

Demographics, aetiology of renal failure, duration of renal replacement therapy (RRT), actual day of the menstrual cycle during the examination, gynaecological history [including menstrual characteristics, number of pregnancies or miscarriages, history of polycystic ovary syndrome (PCOS), family history of premature menopause], and medications potentially (but not definitively) related to AMH concentrations (contraceptives (Bentzen et al., 2012; Deb et al., 2012), prednisolone (Ubaldi et al., 2002) and cyclophosphamide (Clowse et al., 2011)) were recorded.

In addition to AMH, we measured a number of other hormones regulating the ovarian function, including follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin, oestradiol and progesterone. Also, serum creatinine, C-reactive protein (CRP), and thyroid hormones blood concentrations were analysed based on literature showing potential associations with AMH (Yarde et al., 2014; Polyzos et al., 2015; Weghofer et al., 2016).

Menstrual cycles were defined as regular when menstrual flow occurred every 21 to 35 days, irregular when menstrual flow occurred less than 21 days or more than 35 days apart, and amenorrhoea was defined as the abnormal absence of menstruation for 90 days or more in accordance with the NICE guideline (NICE 2014). The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was used for calculation of estimated glomerular filtration rate (eGFR) (Levey et al., 2009).

Study procedures

The blood samples were collected on a random day during the menstrual cycle and then anonymised and centrifuged. In the women on HD, blood samples were obtained before a HD session.

For measurement of AMH, 3mL serum aliquots for each patient were stored at -80^{o}C. AMH was measured on first thaw of stored samples using an automated method on a clinically validated platform (e411, Roche Diagnostics, Burgess Hill, UK) (Gassner and Jung 2014). The assay was calibrated and quality controlled using the manufacturer's reagents. Detection limit was 0.01ng/mL and the coefficient of variation between runs for two levels of control ran at <8%. All AMH samples (including controls) were measured in the same laboratory by the same laboratory-developed test methods in a single run, and all values can therefore be compared uniformly.

All other biochemical parameters were measured using standard assays, in a National Health Service clinical biochemistry department.

Statistical analysis

We examined differences in demographic, clinical factors, and biochemical parameters stratified by renal failure group. Continuous variables were expressed with means and standard deviations (SD) or medians and interquartile ranges (for non-parametric data), and analysed using parametric and non-parametric tests as appropriate. Categorical variables were reported as frequencies and percentages and proportions compared by chi-squared or Fisher's exact tests.

For all comparisons, values of AMH were log transformed to normalise their distribution and were analysed as continuous variables.

One-way ANOVA was performed in the transformed data to examine the percentage change in AMH between renal failure groups. Means (and SD) obtained from the logged values were exponentiated (back transformed) and are therefore the geometric means, which were derived after subtraction of the constant.

Correlation analyses were performed to assess the relationship between log transformed AMH and each predictor variable. Variables tested were age, weight, alcohol and smoking history, eGFR, renal failure group, cause of established renal failure (ERF), biochemical parameters (reproductive hormones, thyroid hormones and CRP), day of menstrual cycle, presence or not of menstrual periods, and medications potentially related to follicular growth (contraceptives, steroids, cyclophosphamide). Factors with p value <0.10 in correlation analyses were tested in linear regression models with log transformed AMH as the outcome variable. Coefficients obtained from linear regression models of the logged values were exponentiated (back transformed) and are therefore ratios of geometric means per unit/category change of the exposure and should be interpreted as proportional change per exposure with a null value of 1.

Mean log transformed AMH levels of young women with renal failure were compared with age-matched healthy references using a two-sample z-test. Z-test was calculated for all patients and in subgroups stratified by age (≤25, 26-30, 31-35, and ≥36 years) and by renal failure group (CKD stages 3-5, HD, and transplant).

The study was designed to enroll 75 participants (25 in each renal failure group), which would be sufficient to detect a significant difference between the healthy controls and women with renal failure assuming the difference in AMH between a healthy and renal failure woman is 0.4ng/mL and SD in each group is 0.7ng/mL, providing power of 80% and probability of type 1 error of 5%(Morel et al., 2013).

For all analyses, a p value <0.05 was considered significant. The IBM SPSS Statistics Package (version 21.0; SPSS, Inc., Armonk, NY) was used for all analyses.

Results

Study population

We measured AMH levels in 77 women of childbearing age who were attending the renal services and fulfilled the study entry criteria. Twenty-six had CKD stages 3-5, 26 were on HD, and 25 had a kidney transplant (Table 1).

Mean age of the enrolled participants was 32.9 (SD 5.4) years, 38 (49.3%) had regular menstrual periods, and approximately a third were on hormonal contraception. More than half had previous successful pregnancies and 18 (23.4%) had at least one miscarriage in the past. Six women (7.8%) had a history of PCOS. No differences of clinical importance were identified between the three groups (Table 1).

Biochemical parameters

Median AMH was higher in HD patients (2.9, IQR 1.1-5.2ng/mL) compared with CKD stages 3-5 patients (1.6, IQR 0.7-2.2ng/mL) and transplant patients (1.5, IQR 1.0-4.2ng/mL) (Table 2). By comparing the logged AMH values by ANOVA, there was significant difference in AMH levels between renal failure groups [$F(2, 74)=3.686$, $p=0.03$]. Tukey post hoc analysis revealed that AMH was significantly higher in HD patients ($p=0.02$) compared with CKD stages 3-5 patients. There was no difference between HD and transplant patients or between CKD and transplant patients. With regard to the rest of the biochemical parameters, there were no differences of note between the three groups, other than thyroid stimulating hormone (TSH) being marginally higher in the CKD group (although still within the 'normal' range) and the transplant patients having better renal function than those with CKD as expected.

From all variables tested, age and renal failure group were associated with AMH on regression analysis (Table 3). When multiple linear regression analysis was performed, there was an average 4% (95%CI 0.02 to 0.06, $p<0.001$) decrease in AMH level per year increase in age when accounting for the renal failure group (Table 3). When compared with women

≤25 years, women older than 36 years had 39% (95%CI 0.08 to 0.62, p=0.04) lower AMH levels (Table 3). Patients on HD had higher AMH levels by 53% (95%CI 0.20 to 0.98, p=0.002) compared with CKD patients, following adjustment for age. No differences were found between the other renal failure groups. The multiple regression model fit was R-squared=0.24 (Table 3). There was no difference in AMH levels between women with menstrual periods vs amenorrhoeic (1.7, IQR 0.8-3.9ng/mL vs 1.5, IQR 1.0-3.0ng/mL, respectively), previously treated with cyclophosphamide vs non-treated (1.5, IQR 0.4-1.5ng/mL vs 1.7, IQR 0.9-3.9ng/mL, respectively), and women on hormonal contraception vs not (1.6, IQR 1.1-3.8ng/mL vs 1.7, IQR 0.8-3.4ng/mL, respectively).

Women with renal failure had 43% lower AMH levels than healthy women (1.7, IQR 0.9-3.8ng/mL vs 3.0, IQR 1.9-5.0ng/mL, p<0.001) (Figure 1). In subgroup analysis, AMH was lower in all age groups in women with renal failure, apart from those aged 26-30 years (≤25yr 1.7 vs 4.0ng/mL, p<0.04; 26-30yr 3.7 vs 3.2ng/mL, not significant; 31-35yr 2.1 vs 2.6ng/mL, p=0.004; ≥36yr 1.0 vs 1.7ng/mL, not significant). When stratified by renal failure group, in comparison with healthy age-matched women controls, AMH was lower in women with CKD stages 3-5 (1.6 vs 3.0ng/mL, p<0.001) and women with a kidney transplant (1.5 vs 3.0ng/mL, p<0.001) but not in women on HD (2.9 vs 3.0) (Figure 2).

Discussion

The data suggest that women of childbearing age with CKD stages 3-5 or a kidney transplant have lower AMH compared with age-matched women without kidney disease and this may contribute to the low fertility rate in this population. Notably, women on haemodialysis had similar AMH levels compared with age-matched controls and this may reflect an intrinsic dysregulation of the granulosa cells leading to higher AMH production or alternatively AMH accumulation in ERF requiring dialysis. In women with renal disease, increasing age was

associated with a reduction in AMH concentrations similar to that observed in the general population. Although pregnancy is extremely uncommon in this patient population, AMH may have a role as a marker of ovarian health in non-dialysis female renal patients pursuing pregnancy. In haemodialysis patients AMH levels seem to be inappropriately high therefore, patients in this group should be excluded from conclusions drawn about the relationship between AMH and ovarian reserve.

To date, AMH has been developed with a wide array of clinical applications (Nelson 2013; Dewailly et al., 2014). These include prediction of the ovarian response to stimulation with exogenous gonadotrophins for in-vitro fertilisation, the duration of the reproductive lifespan and diagnosis of premature ovarian insufficiency, disorders of sex development and PCOS (Seifer et al., 2002; Stubbs et al., 2005; Josso et al., 2012; Iliodromiti et al., 2013; Rey et al., 2013; Tehrani et al., 2013; La Marca and Sunkara 2014). AMH has also been used for the assessment of gonadotoxicity of cancer therapy, monitoring of granulosa cell tumors to detect residual or recurrent disease, and assessing the loss of the ovarian tissue secondary to ovarian surgery (La Marca and Volpe 2007; Geerts et al., 2009; Anderson and Wallace 2013). Women with renal failure and childbearing potential are a diverse group of patients with complex pathologies where AMH may provide valuable insight, especially to those considering future pregnancy.

About 3% of women of childbearing age are affected by renal disease (Williams and Davison 2008). However, the incidence of pregnancy in women with CKD stages 3-5 is difficult to determine as it is not routine practice to measure kidney function in pregnant women in the United Kingdom, unless there is some other indication. Furthermore, kidney function is difficult to interpret during pregnancy as GFR increases. The frequency of conception among women of childbearing age undergoing RRT ranges from 0.3 to 1.5% per year (Holley et al., 1997). For transplant patients the unadjusted pregnancy rate is 33-45 per 1000 women (Gill et

al., 2009; Stoumpos et al., 2016) compared with more than 100 per 1000 women in the general population.

Circulating AMH concentrations reflect the functional ovarian reserve and indirectly the number of residual primordial follicles within the ovary. As a consequence of this intimate relationship with the ovarian reserve, a decline in AMH may indicate both physiological and premature aging of the gonads (Kalaiselvi et al., 2012; Younis 2012). Applicable to the population with renal failure, in women with moderate to severe CKD (stages 3-5), the risk of complications to mother and fetus with pregnancy is high enough that some advocate against pregnancy or to postpone until they receive a kidney transplant. Similarly, female kidney transplant recipients are traditionally counselled to wait one to two years after transplantation before conceiving (McKay and Josephson 2008). This postponement frequently leads to women with renal disease attempting to have children during a period where female fertility is already in decline due to ageing. Furthermore, menopause in women with renal failure occurs 4.5 years earlier than in healthy women (Weisinger and Bellorin-Font 2004) leaving them with fewer potential childbearing years. Nonetheless, not all cases are clear-cut and there is a number of women with either advanced renal disease or a kidney transplant in whom assessment of AMH may be useful to guide pregnancy planning. For example, a young woman with normal AMH values would potentially have more time to deal with any underlying medical issues. In contrast, women with a borderline or low AMH, indicative of a diminished ovarian reserve, may be counselled that fertility preservation may be advantageous.

Women on HD were found to have higher AMH concentrations compared with the other two renal failure groups and this was an unexpected finding. Impaired glomerular filtration could be a potential mechanism although AMH has a molecular weight of 140kD, which is too large to cross the basement membrane of the glomerular capillaries. On the other hand, AMH

is of similar size to other molecules perceived to be uraemic ‘toxins’ (Duranton et al., 2012) (i.e. fibrinogen, a2-macroglobulin) and although their concentrations are not directly associated with glomerular function they are increased in dialysis, so accumulation of AMH in dialysis is plausible. Uraemia is known to interfere with the metabolism and regulation of a number of endogenous hormones; however, the direct impact of uraemia in ovarian follicles and AMH levels is not clear. We hypothesise that high AMH levels during dialysis are probably related to the follicular arrest during the selection process of the dominant follicle, through a negative interaction between AMH and FSH (Grossman et al., 2008). If so, AMH significantly decreases FSH-induced oestradiol production, which leads to disruption of normal antral follicular development and maturation, similarly to what is happening in women with PCOS (Agarwal et al., 1996). In accordance with this, oestradiol levels were lower in women on HD compared with the other two renal failure groups, although the difference was not significant. In a study of 186 young healthy Danish women (Hagen et al., 2012) that were followed until they conceived or for six menstrual cycles, the fecundability ratio (FR) (i.e., the monthly probability of conceiving) in women with low AMH levels was similar to women with medium AMH levels (FR 0.81; 95% CI 0.44–1.40) but women with high AMH levels had reduced FR (FR 0.62; 95% CI 0.39–0.99). This supports our findings, where high AMH levels may represent women with conditions of anovulation. In a recently published study (Sikora-Grabka et al., 2016), women on HD had similar AMH concentrations with healthy women and this is similar to our findings. However, when women on HD were stratified into those with regular menstrual cycles vs not, the regularly menstruating women on HD had significantly lower serum AMH concentration compared with the healthy controls. In our study there was no difference in AMH concentrations between women on HD with regular, irregular periods or amenorrhoea; however, the numbers were very small (n=11, n=8 and n=7, respectively). In the same study, AMH concentrations were found to gradually

decrease from pre- to 6 months post-transplantation and this may reflect the transition from abnormally high AMH concentrations during dialysis to lower 'normal' concentrations post-transplantation.

Our study has a number of strengths including the use of a cohort of young, well-phenotyped women with renal failure, distinct clinical categorisation of disease progression and that we could compare them with a large cohort of age-matched healthy controls. We performed extensive biochemical characterisation, including use of a robust automated AMH assay. We do however, acknowledge some limitations: information on menstrual cycle characteristics were not easy to ascertain in all cases, especially in women with scanty menstrual periods; however, AMH has been shown to be stable throughout the menstrual cycle and in women with irregular cycles random sampling is inevitable. We had limited power to assess the effects of cyclophosphamide or the combined oral contraceptive pill, but overall AMH was reduced in keeping with previous larger studies. In women with renal failure other markers of ovarian reserve, such as antral follicle count (AFC) which is an ultrasound biomarker of follicle number may be a better predictor of oocyte yield than AMH. We were unable to perform transvaginal ultrasound examination and AFC measurement to provide additional assessment of the ovarian reserve; however, AFC is not routinely available and is known to exhibit substantial intra- and interobserver variability. Lastly, we accept that no causal inferences on the effect of renal failure on AMH levels can be made, with prospective longitudinal studies best placed to address this.

We demonstrate that in women of reproductive age with CKD stages 3-5 or a renal transplant, AMH exhibits a similar age-related decline but age-specific values are lower than equivalent healthy controls. For women on HD circulating AMH concentrations are higher, suggesting a disruption of folliculogenesis or impaired clearance. AMH evaluation may be a useful biochemical test in estimating the reproductive lifespan in women with renal disease

not treated with dialysis, pursuing preconception counselling. However, it is important to note that AMH is only a marker of ovarian reserve and should be used as an adjuvant tool. Conception can be achieved with low AMH levels therefore, one should be careful about the interpretation of AMH as a prognostic marker for conception success. Further research is warranted to investigate the direct impact of uraemia on ovarian follicles and AMH production.

Disclosure

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Figure 1. Log AMH levels in young women with CKD (n=77) compared with AMH levels in a cohort of 600 age-matched healthy women ($p<0.001$, two-sample z-test). Data are log-transformed.

AMH, anti-Müllerian hormone; CKD, chronic kidney disease.

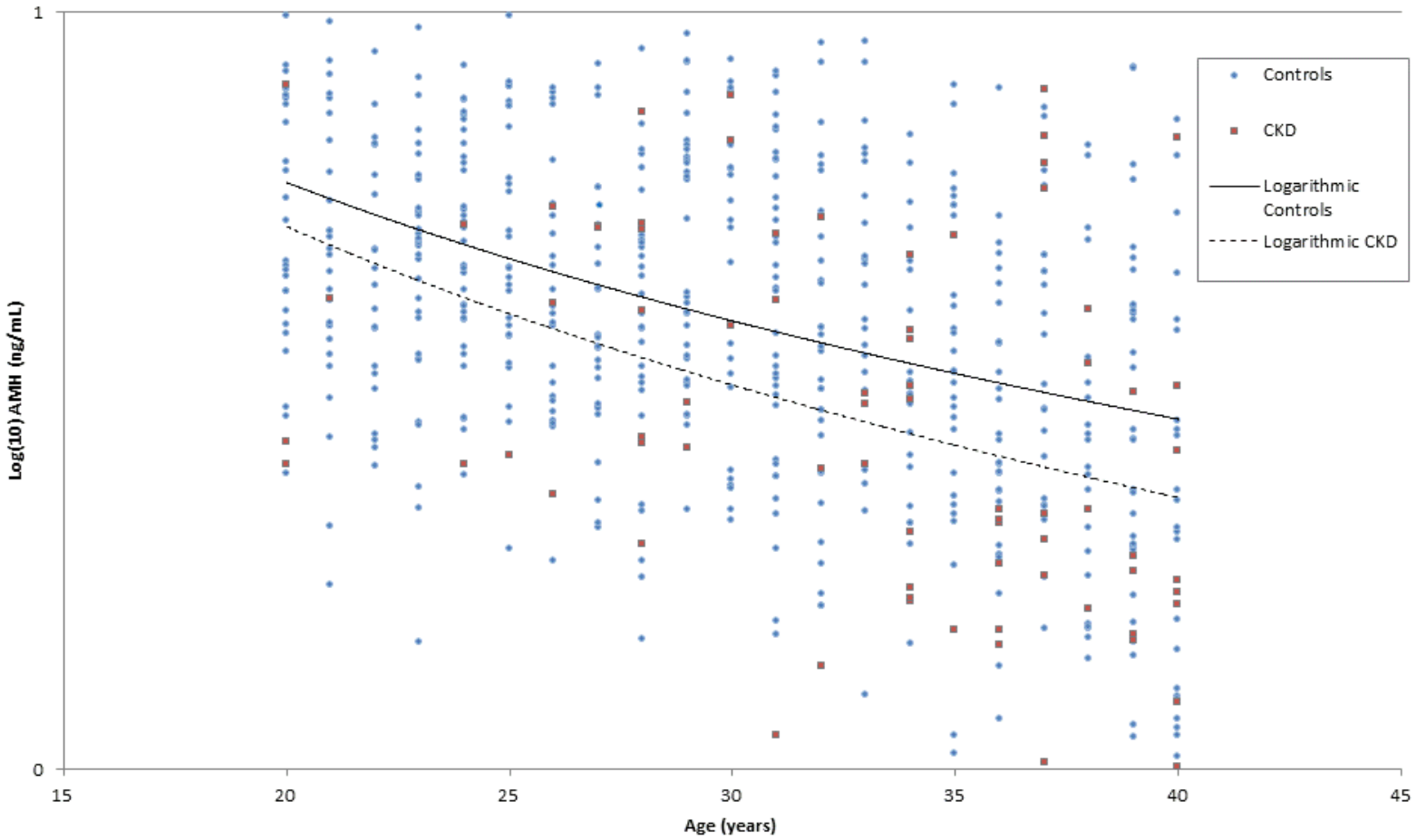

Figure 2. Age-related distribution of serum log AMH levels in women of reproductive age with CKD stages 3-5 (n=26), on HD (n=26), kidney transplant recipients (n=25) and healthy controls (n=600). Data are log-transformed.

AMH, anti-Müllerian hormone; CKD, chronic kidney disease; HD, haemodialysis.

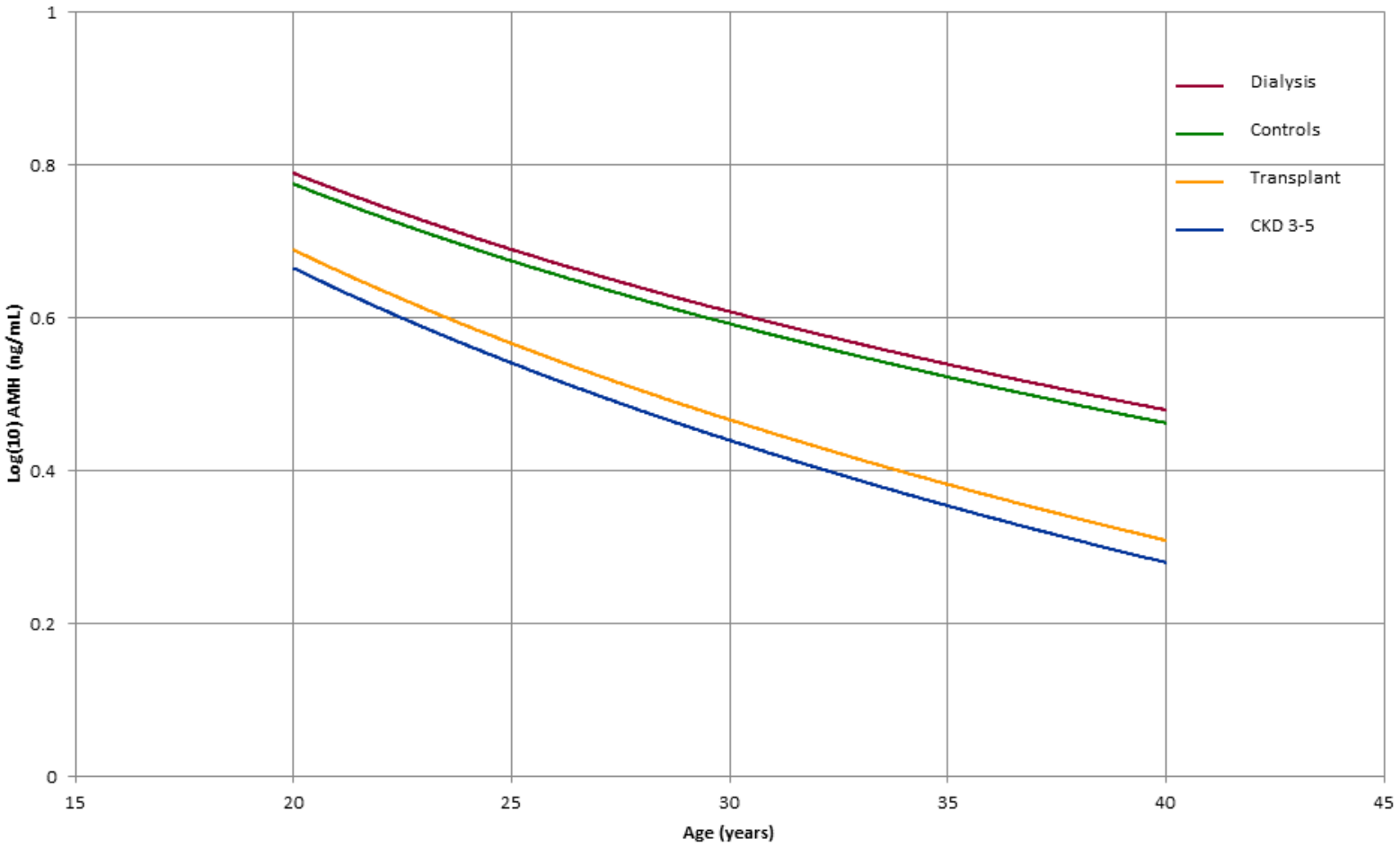

Table 1. Baseline characteristics stratified by renal failure group					
	All patients (n=77)	CKD (n=26)	HD (n=26)	Transplant (n=25)	*p-value*[a]
Age (years), mean (SD)	32.9 (5.4)	33.4 (5.4)	34.0 (4.9)	31.2 (5.8)	NS
Weight (kg), mean (SD)	68.1 (17.8)	71.2 (18.8)	66.8 (15.3)	66.1 (19.4)	NS
Ethnic origin					
Caucasian, n (%)	66 (85.7)	20 (76.9)	26 (100.0)	20 (80.0)	0.04
Asian, n (%)	11 (14.3)	6 (23.1)	0 (0.0)	5 (20.0)	
Smoking[b], n (%)	25 (32.5)	7 (26.9)	12 (46.2)	6 (24.0)	NS
Alcohol[c], n (%)	40 (51.9)	17 (65.4)	11 (42.3)	12 (48.0)	NS
Cause of ERF					
Glomerulonephritis, n (%)	31 (40.3)	10 (38.5)	9 (34.6)	12 (48.0)	NS
Congenital renal dysplasia/reflux, n (%)	14 (18.2)	3 (11.5)	5 (19.2)	6 (24.0)	
Diabetes, n (%)	12 (15.6)	6 (23.1)	5 (19.2)	1 (4.0)	
Other[d], n (%)	20 (26.0)	7 (26.9)	7 (26.9)	6 (24.0)	
Years on RRT, median (IQR)	4.3 (1.6, 11.3)		2.8 (0.6, 7.5)	8.7 (3.1, 12.0)	0.03
Menstrual periods					
Amenorrhoea, n (%)	15 (19.5)	5 (19.2)	7 (26.9)	3 (12.0)	NS
Regular, n (%)	38 (49.4)	12 (46.2)	11 (42.3)	15 (60.0)	
Irregular, n (%)	24 (31.2)	9 (34.6)	8 (30.8)	7 (28.0)	
Previous pregnancies, n (%)	46 (59.7)	15 (57.7)	17 (65.4)	14 (56.0)	NS
Previous miscarriages, n (%)	18 (23.4)	8 (30.8)	5 (19.2)	5 (20.0)	NS
Polycystic ovary syndrome, n (%)	6 (7.8)	2 (7.7)	1 (3.8)	3 (12.0)	NS
Family history of premature menopause[e], n (%)	3 (5.5)	3 (16.7)	0 (0.0)	0 (0.0)	0.04
Medications related to follicular growth					
Hormonal contraception, n (%)	29 (37.7)	12 (46.2)	8 (30.8)	9 (36.0)	NS
Steroids, n (%)	37 (48.1)	5 (19.2)	8 (30.8)	24 (96.0)	<0.001
Previous cyclophosphamide, n (%)	5 (6.5)	2 (7.7)	1 (3.8)	2 (8.0)	NS

[a] ANOVA or chi-squared test or Fisher's exact test or Mann-Whitney U test where appropriate

[b] Current or previous vs never

[c] Occasionally (<14units/week) vs never

[d] Polycystic kidney disease (n=6), unknown aetiology (n=6), haemolytic uremic syndrome (n=2), post-acute kidney injury (n=2), malignant hypertension (n=1), tubulointerstitial nephritis (n=1), amyloidosis (n=1), nephronophthisis (n=1)

[e] Excludes 22 patients with missing values

CKD, chronic kidney disease; HD, haemodialysis; SD, standard deviation; NS, not statistically significant; ERF, established renal failure; RRT, renal replacement therapy; IQR, interquartile range.

Table 2. Biochemical parameters stratified by renal failure group					
	All patients (n=77)	CKD (n=26)	HD (n=26)	Transplant (n=25)	*p-value*[a]
eGFR[b], mL/min/1.73m^2; mean (SD)	50.8 (27.6)	34.4 (15.3)		67.2 (27.7)	<0.001
<45[b], n (%)	26 (51.0)	19 (73.1)		7 (28.0)	0.005
≥45, n (%)	25 (49.0)	7 (26.9)		18 (72.0)	
Day of menstrual cycle[c], median (IQR)	12 (6-22)	16 (5-24)	12 (4-22)	12 (9-17)	0.77
Anti-Müllerian hormone, ng/mL; median (IQR)	1.7 (0.9, 3.8)	1.6 (0.7, 2.2)	2.9 (1.1, 5.2)	1.5 (1.0, 4.2)	0.03[d]
Anti-Müllerian hormone, ng/mL; geometric mean (SD)[e]	1.9 (0.7)	1.4 (0.5)	2.6 (0.8)	1.8 (0.8)	
Follicle-stimulating hormone, IU/L; median (IQR)	4.1 (2.5, 6.0)	5.1 (3.7, 6.0)	3.8 (2.6, 5.6)	3.0 (2.5, 6.3)	0.37
Luteinising hormone, IU/L; median (IQR)	6.5 (3.8, 10.0)	6.8 (3.8, 8.3)	5.0 (3.6, 11.5)	5.6 (3.8, 12.4)	1.00
Prolactin, mIU/L; median (IQR)	360 (247, 563)	306 (243, 469)	444 (345, 652)	315 (206, 544)	0.08
Oestradiol, pmol/L; median (IQR)	242 (102, 395)	226 (113, 326)	130 (82, 317)	320 (132, 585)	0.12
Progesterone, nmol/L; median (IQR)	1.3 (1.0, 4.7)	1.1 (1.0, 4.8)	1.6 (1.2, 4.2)	1.0 (1.0, 3.2)	0.45
Thyroid stimulating hormone, mIU/L; median (IQR)	1.5 (0.9, 2.1)	1.8 (1.4, 2.4)	1.3 (0.9, 1.7)	1.1 (0.7, 2.0)	0.03
Free thyroxine, pmol/L; median (IQR)	13.2 (12.4, 14.9)	12.9 (12.3, 14.6)	13.2 (12.4, 14.1)	14.0 (12.7, 15.5)	0.38
C-reactive protein, mg/L; median (IQR)	2.5 (1.0, 6.3)	2.0 (1.0, 5.0)	3.5 (1.0, 9.8)	2.0 (1.0, 5.0)	0.37

[a] T-test or chi-squared test or Kruskal-Wallis test or ANOVA where appropriate

[b] Excludes patients on dialysis

[c] Excludes 15 women with amenorrhoea and 8 women with menstrual cycles >35 days

[d] P-value was obtained by comparing logged values

[e] Geometric means were derived from logged AMH values after back transformation

CKD, chronic kidney disease; HD, haemodialysis; eGFR, estimated glomerular filtration rate; SD, standard deviation; IQR, interquartile range.

Table 3. Covariates associated with AMH levels						
Covariate	**Simple linear regression**			**Multiple linear regression**		
	Ratio of geometric means (Bootstrap 95%CI)	***p*-value**	**R-square**	**Ratio of geometric means (Bootstrap 95%CI)**	***p*-value**	**R-square**
Age (each additional year)	-0.04 (-0.06 to -0.02)	0.001	0.15	-0.04 (-0.06 to -0.02)	<0.001	0.24
Renal failure group			0.09			
CKD (reference)	1.0	-		1.0	-	
HD	0.49 (0.16 to 1.00)	0.009		0.53 (0.20 to 0.98)	0.002	
Transplant	0.16 (-0.13 to 0.53)	0.31		0.06 (-0.18 to 0.36)	0.70	
Age group			0.16			
≤25 (reference)	1.0	-				
26-30	0.04 (-0.34 to 0.60)	0.88				
31-35	-0.20 (-0.49 to 0.22)	0.36				
≥36	-0.39 (-0.62 to -0.08)	0.04				
AMH, anti-Müllerian hormone; CI, confidence interval; CKD, chronic kidney disease; HD, haemodialysis.						