

## Original Research

# Assessment of semen parameters and Anti-müllerian hormone level in patients with chronic kidney disease on hemodialysis: a case control study

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## Abstract

**Background and Objective:** Chronic kidney disease (CKD) is a common health problem which is accompanied by considerable morbidity and mortality. Anti-müllerian hormone (AMH) is one of the hormones that are affected by CKD. The objective was to assess semen parameters and AMH level in CKD patients on hemodialysis.

**Material and methods:** This study was a case control study of 20 patients with stage 4 and 5 CKD on hemodialysis, attending Nephrology Outpatient Clinic and Renal Dialysis Unit of Suez Canal University hospital and 20 healthy fertile controls. Full history, clinical examination, kidney function tests and semen analysis were performed. AMH level in serum and seminal plasma was measured using ELISA.

**Results:** The mean AMH level in serum of case group was  $3.35 \pm 2.43$  ng/mL versus  $4.13 \pm 3.17$  ng/mL in the controls respectively ( $p > 0.05$ ) while mean AMH level in semen of the case group was  $2.13 \pm 2.64$  ng/mL compared to  $9.37 \pm 3.79$  ng/mL in controls respectively ( $p < 0.05$ ). There was a positive correlation between AMH in seminal plasma and sperm count, total sperm motility, progressive sperm motility and sperm viability, while the AMH level in serum was insignificantly related.

**Conclusion:** Semen parameters in CKD patients on hemodialysis were significantly poorer than normal controls AMH in seminal plasma was significantly decreased in CKD patients (stage 4-5 on hemodialysis) and significantly correlated with the semen parameters.

## Keywords

CKD; Serum AMH; Seminal AMH; Semen parameters

## 1. Introduction

Chronic kidney disease (CKD) is a common universal pathological condition that is characterized by either impaired renal functions or complete kidney damage or failure for at least three months regardless of the etiology [1].

Diagnosis of kidney damage is reliant on proteinuria, as a loss of more than 150 mg of protein and 30 mg of albumin in urine per day indicates damage of the kidneys due to increased glomerular permeability facilitating macromolecules filtration that shouldn't be excreted in urine [2]. CKD has five

stages classified according to estimated GFR, from stage one with normal kidney function to stage five with severe kidney damage and GFR lower than  $15 \text{ mL/min/1.73 m}^2$  [3].

There are numerous pathophysiological alterations that appear in the body in conjugation with declining renal function in male patients with CKD. Among these alterations, the reproductive and erectile functions are impaired [4].

Males with renal failure usually show evidence of infertility due to various factors including hypo-gonadism, direct harm to the spermatogenesis process with spermatotoxicity and late maturation arrest which causes azoospermia or oligo-

zoospermia [5].

For patients with CKD, male fertility potential and semen quality are significantly affected in comparison to healthy fertile or infertile men without CKD [6], although some potential fertility improvement occurs after renal transplantation, but is still not satisfactory for achieving normal seminograms [7].

Anti-müllerian hormone (AMH) is a part of the classical pathway for the induction of the male phenotype [8]. In males, AMH is produced from testicular immature sertoli cells into the serum and seminal fluid and it causes regression of müllerian ducts in the male fetus as a part of the sexual differentiation process [9].

AMH plays a critical paracrine role in the regulation of Leydig cell development and testosterone biosynthesis [10]. The mechanism of action and the chief physiological function of AMH in adult males is not completely understood [11], even though it is considered a functional indicator of immature sertoli cells. AMH in high levels during early life will lead to increased germ cells support in adulthood by mature sertoli cells; consequently, levels of AMH might be related to male fertility or infertility [12].

Mirroring the impaired testicular functions, men with CKD on hemodialysis have a 60% lower level of serum AMH in comparison to the healthy fertile people [11]. Serum AMH levels were found to be lower in CKD by 30% in stages one to four and by 70% in stage five CKD compared to normal people, which indicates that sertoli cell functions are altered among those patients [3]. No previous studies compared the level of AMH level in serum and seminal plasma simultaneously in the same patient with chronic kidney disease on hemodialysis in relation to fertile controls. The aim of this work was to measure AMH levels in serum and seminal plasma of CKD patients on hemodialysis, as well as to evaluate a change in semen parameters in patients with CKD in comparison to controls, and to appraise the predictive value of serum and semen levels of AMH in male fertility diagnosis.

## 2. Patients and methods

### 2.1 Patients

This study was carried out as a case control study on 20 male patients suffering from CKD. Patients had been recruited from the Nephrology Outpatient Clinic, Faculty of Medicine, Suez Canal University hospitals, Ismailia, Egypt; during the period from January 2018 to January 2019.

Twenty patients with CKD who agreed to participate in the study were chosen by simple random sampling, from patients attending the Renal Dialysis Unit, Nephrology Department, and Suez Canal University Hospital.

Causes of CKD in studied patients were diabetes mellitus, polycystic kidney disease, glomerulonephritis, chronic neglected urinary tract infections, obstructive uropathies and chronic medications.

Inclusion criteria incorporated male patients aged more than 18 years of age and suffering from CKD grades 4 & 5 and on hemodialysis, either fertile or not. Exclusion criteria

included patients with CKD grades 1, 2 & 3, patients with testicular atrophy, cryptorchidism, aspermia, obstructive azoospermia or chronic orchitis, patients with co-morbidities interfering with fertility or testicular functions, patients on immunosuppressive therapies and patients with a history of genital surgeries or that had received medical treatment for infertility in the last 3 months prior to the study.

The control group included 20 fertile subjects (meaning the subjects had the ability to impregnate their wives after less than 12 months of regular sexual intercourse without the use of contraceptives and without the use of assisted reproduction) and with normal kidney functions (based on history taking and clinical examination). Controls were chosen by a simple random sample technique from relatives of CKD patients or attendees of the Andrology Clinic with other complaints rather than infertility. Patients had been chosen to be fertile to exclude other factors that affect AMH production.

This study was approved by the Institutional Review Board and the Ethics Committee. All contributors signed an informed consent form that included study aims, objectives and applications at the beginning of the study.

### 2.2 Methods

All of the studied subjects were exposed to complete medical history (personal, present, fertility, sexual, family and past medical and surgical history) and complete physical examinations (general and genital examinations).

Patients with CKD chosen for the study had been scheduled for hemodialysis three times per week. Duration of each session was at least 4 hours. Diagnosis of stage 4 and 5 CKD was confirmed if the estimated Glomerular Filtration Rate (eGFR) ranged from 15 to 29 mL/min/1.73 m<sup>2</sup> for stage 4 and less than 15 mL/min/1.73 m<sup>2</sup> for stage 5 CKD. eGFR was estimated by measuring serum creatinine levels using the Modification of Diet in Renal disease (MDRD) study and Cockcroft-Gault equations (Normal eGFR range from 90–120 mL/min/1.73 m<sup>2</sup>) [13].

Dialysis regimen was different between one patient to the other according age, weight, general condition, duration of disease, laboratory investigations and associated diseases and medications. Dialysis sessions were done thrice weekly. Average dialysis prescriptions in most of studied patients were; Time 3–4 hours, dialyzer: smaller, low flux dialyzer with low urea clearance and low KoA, blood flow: 200–300 mL/min, dialysate flow: 300–500 mL/min, volume removal: according patient volume status and concentrate: according upon patient laboratory values.

Semen samples were collected after getting informed consent from the patients and controls. The samples were collected by masturbation in a wide-mouthed graded container after 4 days of sexual abstinence. Patients were instructed to collect the full amount of semen ejaculated into the container. Semen analysis was performed for all of the study participants in the Andrology, Sexology and STDS Outpatient Clinic, Faculty of Medicine, Suez Canal University Hospital. The results of the semen analysis were assessed according to the

**TABLE 1. Frequency distribution of cases and controls regarding age, weight, renal functions, semen parameters and AMH levels.**

	Case group		Control group		T test	
	Mean ± SD	Range	Mean ± SD	Range	p value	Sig.
Age (Years)	37.30 ± 10.07	22–54	31.50 ± 6.81	24–45	0.039	S
Weight (kg)	69.20 ± 3.29	50–84	83.88 ± 12.97	60–105	0.187	NS
BMI	24.45 ± 4.8	18–31	28.8 ± 6.1	20–35	0.247	NS
Serum creatinine (mL/dL)	10.55 ± 3.28	2.80–15	1.09 ± 0.16	0.87–1.40	<0.001	S
eGFR (mL/min)	12.26 ± 7.58	7.09–34	112.57 ± 25.7	82.30–157.71	<0.001	S
Volume (mL)	2.11 ± 3.26	0.5–5	3.15 ± 0.61	2–10	<0.001	S
Count per mL (million)	21.70 ± 31.78	0.5–100	74.50 ± 17.21	28–200	<0.001	S
Total motility (%)	17.60 ± 15.35	0–45	72.50 ± 8.35	60–85	<0.001	S
Progressive motility (%)	7.70 ± 9.66	0–27	48.50 ± 9.75	35–65	<0.001	S
Immotility (%)	78.40 ± 20.18	20–100	22.50 ± 8.35	10–35	<0.001	S
Viability (%)	49.17 ± 24.01	10–80	84 ± 7.54	70–95	<0.001	S
Abnormal forms (%)	82.86 ± 8.25	70–95	57.50 ± 8.35	40–70	<0.001	S
AMH in serum (ng/mL)	3.35 ± 2.43	0.81–7.41	4.13 ± 3.17	0.97–11.60	0.386	NS
AMH in semen (ng/mL)	2.13 ± 2.64	0.04–8.66	9.37 ± 3.79	2–14.42	<0.001	S

eGFR, estimated Glomerular Filtration Rate; BMI, Body Mass Index; AMH, Anti-Müllerian Hormone.

World Health Organization 2010 reference values [14].

Anti-müllerian hormone level was measured in serum and seminal plasma of patients and controls by ELISA using a commercial kit (Ansh Labs Ultrasensitive AMH/MIS ELISA, Webster, TX, USA). Measurement unit was ng/mL and detection range was 0.084–14.42 ng/mL.

### 2.3 Statistical analysis

Data that was collected were coded, entered and analyzed using Microsoft Excel software. Data was then imported into Statistical Package for the Social Sciences (SPSS 20.0) software program (SPSS Inc., Chicago, IL, USA) for analysis. T test was used for quantitative data and p value had been set at  $\leq 0.05$  for significant results. Pearson's correlation coefficient was used in correlations.

### 3. Results

The mean age was higher in the patients group than the control group, while weight and BMI were higher in controls than patients. Regarding semen parameters in cases and in the control group, all semen parameters including semen volume, sperm count per mL, total motility, progressive motility, viability and normal morphology were significantly lower in CKD patients in comparison to normal fertile population. Interpretation of results of semen parameters in the patients group revealed that only 15% of patients with CKD had normal semen (Table 1).

Mean level of AMH in serum of patients with CKD was lower in the patients group in comparison to the control group ( $p > 0.05$ ) while the mean level of AMH in seminal plasma was significantly lower in the patients group in comparison to the control group (Table 1).

Correlation between AMH serum level and semen parameters revealed that there was no significant statistical relationship between AMH level and all semen parameters both in cases and in controls ( $p > 0.05$ ). AMH level in semen

of cases was significantly related to sperm count per mL, total motility, progressive motility viability and abnormal morphology ( $p < 0.05$ ), while in controls it was significantly related to sperm count per mL, total motility, progressive motility, immotility and viability ( $p < 0.05$ ) (Table 2).

AMH level in seminal plasma can accurately identify cases with abnormal semen parameters as indicated by a significant coverage of area under the curve (AUC = 0.95,  $p < 0.001$ ) while AMH in serum can't identify cases with abnormal semen parameters as indicated by a non-significant coverage of area under the curve (AUC = 0.54,  $p = 0.673$ ) (Table 3).

### 4. Discussion

Male patients with CKD usually have a prominent interruption of the hypothalamic-pituitary-gonadal axis that increases in severity in conjunction with worsening kidney functions, resulting in hormonal disturbances and deterioration in testicular functions [15, 16].

AMH is a hormone secreted in males only from sertoli cells in the serum and seminal fluid. The major physiological function of AMH in the adult male appears to be the autocrine and paracrine control of testicular functions [17].

AMH in the testis is secreted by sertoli cells both apically into seminiferous tubules and basally towards the interstitium and circulation. After puberty, levels of AMH in seminal plasma was found to be higher than serum levels which can be explained by the preferential release of AMH by the apical pole of the sertoli cells towards the lumen of the seminiferous tubules and hence to seminal plasma [18].

In CKD the inflammatory milieu, the uremic milieu, hormonal disturbances and deterioration of testicular functions may influence AMH secretion from the sertoli cells [19].

In the current work, mean serum and semen levels of AMH was higher in normal fertile people without CKD than patients with CKD on hemodialysis, with significant difference regarding semen levels. These results regarding serum level

**TABLE 2. Correlations between Anti-müllerian hormone level in serum and semen of cases and controls and different semen parameters.**

		Volume	Count	Total motility	Progressive motility	Immotility	Viability	Abnormal forms
Serum AMH in	r	0.052	-0.116	0.377	0.328	-0.111	-0.150	0.492
Cases	p value	0.838	0.626	0.101	0.159	0.641	0.643	0.074
Serum AMH in	r	0.213	0.202	0.061	0.293	-0.114	-0.480	-0.387
Controls	p value	0.368	0.392	0.799	0.209	0.631	0.329	0.091
Semen AMH in	r	-0.164	0.606	0.401	0.459	0.151	0.653	-0.726
Cases	p value	0.515	0.005*	0.038*	0.042*	0.526	0.021*	0.003*
Semen AMH in	r	-0.383	0.478	0.459	0.575	-0.614	0.466	-0.076
Controls	p value	0.096	0.033*	0.042*	0.008*	0.004*	0.038*	0.750

AMH, Anti-Müllerian Hormone; r, Pearson's correlation coefficient; if  $r = 0-0.19$  is regarded as very weak correlation,  $r = 0.2-0.39$  as weak correlation,  $r = 0.40-0.59$  as moderate correlation,  $r = 0.6-0.79$  as strong correlation,  $r = 0.8-1$  as very strong correlation, \* = ( $p$  value  $< 0.05$ ) regarded as significant.

**TABLE 3. Sensitivity and specificity of Anti-müllerian hormone level in serum and semen for detection of cases with abnormal semen parameters.**

	AUC	95% CI	Cutoff point	Sensitivity	Specificity	PPV	NPV	p value	Sig.
AMH in serum	0.54	0.375 to 0.698	$\leq 1.04$	30	90	75	56.2	0.673	NS
AMH in semen	0.95	0.831 to 0.994	$\leq 3.97$	90	90	90	90	$< 0.001$	S

AMH, Anti-Müllerian Hormone; AUC, Area under the curve; CI, Confidence Interval; PPV, Positive predictive value; NPV, Negative predictive value.

of AMH are different from Eckersten *et al.* [11], who studied 20 male patients on hemodialysis and 144 proven fertile males as a control group to determine serum levels of AMH using a two-step immunometric ELISA. They found that the mean value of AMH was significantly decreased ( $19.5 \pm 13.3$  pmol) when compared to the control group ( $47.3 \pm 25.9$  pmol), which meant a decrease by close to 60% when compared with controls. This discrepancy may be attributed to the Beckman Coulter assay that was used on the unadulterated samples, which has reported complement interference that leads to false low results, unlike Ansh Labs Ultra Sensitive AMH assay which did not have any interference. There are no available studies comparing semen AMH levels in CKD between patients and controls.

In the current study we found that semen parameters were significantly higher in the control group than the patients group, and only 15% of patients with CKD had normal semen. These results concerning the decrease in sperm count and motility are in agreement with Prem *et al.* [20] who studied 19 patients with end stage renal disease on dialysis to evaluate the impact of maintenance of hemodialysis on the reproductive potential. They found that there was complete azoospermia, severe oligozoospermia (sperm density  $< 5 \times 10^6$  mL), moderate oligozoospermia (a sperm density of  $< 5 \times 10^6$  mL to  $< 20 \times 10^6$  mL), and a decrease in the mean percentage of motility ( $13.5 \pm 9.6\%$ ) in semen analysis of patients with chronic renal failure, which showed that disturbance in kidney function influences male sperm parameters.

Eckersten and colleagues studied 30 males with CKD stage one to four in addition to 18 normal controls to determine semen parameters in CKD patients who were not yet on dialysis. They found that the mean sperm concentration was significantly lesser among men with stages 3-4 CKD ( $(42 \pm 29) \times 10^6$ /mL) in comparison to controls ( $(88 \pm$

$42) \times 10^6$ /mL). These results are in agreement with the current study results concerning the significant decrease in the mean sperm count ( $21.70 \pm 31.78$  million) in the CKD group of patients compared to the control group ( $74.50 \pm 17.21$  million) [21].

Regarding correlation between AMH serum levels and semen parameters in the current study, no significant correlation was found between AMH serum levels and sperm count, total motility, progressive motility and viability in the CKD patients. These results are in agreement with Aksglaede *et al.* [22] who studied 970 young Danish men to clarify the relation between the AMH serum levels and semen parameters. Serum AMH and semen parameters (semen volume, sperm concentration, and motility and morphologically normal sperms) were measured. They reported that there is no significant correlation between serum AMH and semen quality, concluding that serum AMH is not useful as a marker of male fertility. This is in contrast to Eckersten and colleagues, who studied 30 male patients with stage 1 to 4 CKD as well as 18 healthy controls to study possible associations between serum AMH level and semen parameters as markers of fertility. They found that AMH levels showed positive significant correlation with the percentage of motile sperms [21].

On the other hand, correlation between AMH levels in seminal plasma and semen parameters in the current study revealed that there was positive significant correlation between AMH levels in seminal plasma and sperm count, total sperm motility, progressive sperm motility and sperm viability in both the CKD group and the control group. These results were in concordance with the study conducted by Andersen *et al.* [23], who reported positive significant correlation between AMH levels in semen and sperm count per mL, total sperm count and progressive sperm motility. In the same

line, Kucera and colleagues observed a significant decrease in the mean value of AMH in seminal plasma in the group with a sperm count <15 million (3.29 ng/mL) compared to the healthy control group (14.21 ng/mL), but there was non-significant decrease in the mean value of AMH in the serum in the group with a sperm count <15 million (4.77 ng/mL) compared to the control group (6.95 ng/mL), and concluded that AMH serum levels didn't correlate with the pathological sperm count but AMH seminal plasma levels positively correlate with the pathological sperm count [24].

Opposing results were reported by Al Qahtani *et al.* [25] who investigated semen AMH levels in normal fertile males and infertile males. They found that the mean AMH level in semen samples from infertile males was  $10.2 \pm 4$  ng/mL versus in control males was  $17.54 \pm 5$  ng/mL. This result was not significantly different, and in addition there was no association between seminal levels of AMH and spermatogenesis.

Limitations of the study were age difference, associated comorbidities, medications, small sample size, difficulty to obtain semen samples from patients, erectile dysfunction, bad psychological status from disease burden or medications and the high cost of AMH measurement.

## 5. Conclusions

Semen parameters in CKD patients on hemodialysis were significantly poorer than normal controls. AMH level in seminal plasma was significantly decreased in patients with CKD on hemodialysis than controls and significantly correlated with semen parameters while AMH in the serum was decreased insignificantly in patients with CKD on hemodialysis than controls and did not correlate with semen parameters. This indicates that AMH level in seminal plasma could be used as a marker of disturbed testicular functions and subfertility in men with CKD (stages 4–5), as it could reflect disturbed sertoli cell functions while AMH in serum could not be considered as a reliable marker.

## Abbreviations

CKD, Chronic kidney disease; AMH, Anti-müllerian hormone; eGFR, estimated Glomerular Filtration Rate; CASA, Computer assisted semen analysis.

## Author contributions

RM, GMS, MA and AFAA planned and designed the study. RM and MA supported considerable contributions to the study conception and design. RM, GMS and MA collected the data. GMS and AFAA implemented the clinical part and investigations. GMS analyzed and interpreted the data. MA drafted the article. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

This study was approved by the Institutional Review Board and the Ethics Committee, Faculty of Medicine, Suez Canal

University, Ismailia, Egypt. All contributors signed an informed consent form included study aims, objectives and applications at the beginning of the study. Approval number was 2956, date 9 September 2016.

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## Conflict of interest

The authors declare no conflict of interest.

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