Study evaluating testosterone deficiency as a cause of anemia and reduced responsiveness to erythropoiesis-stimulating agents in men on maintenance hemodialysis

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Introduction
Chronic kidney disease (CKD) is a worldwide disease that is classified into five stages according to the glomerular filtration rate and presents through a variety of symptoms and signs. Anemia is one of the first signs of kidney dysfunction. The most common causes of anemia in CKD are erythropoietin (EPO) hormone deficiency and iron deficiency. Anemia and hyporesponsiveness to erythropoietin-stimulating agents (ESAs) are commonly observed in CKD patients and are associated with increased morbidity, mortality, and a significant healthcare economic burden. Although testosterone deficiency is a prevalent condition in men with CKD, it has so far received relatively little attention in practice. Testosterone stimulates erythropoiesis through the production of hematopoietic growth factors and possible improvement of iron bioavailability.

Aim
The aim of this study was to evaluate serum testosterone levels in patients on maintenance hemodialysis (MHD) and correlate its level with anemia and response to ESAs therapy.

Patients and methods
This study included 40 male patients from dialysis units, where they were divided equally into group A, group taking ESAs, and group B, group not taking ESAs (EPO-naive group). Another 20 men were included in group C (control group). All groups were subjected to a full assessment of history, full clinical examination, and laboratory investigations to exclude all possible causes of anemia.

Results
This study showed that in group A, 75% of the participants were anemic, whereas in group B, 100% of the participants were anemic, with a higher degree of anemia. The testosterone level was slightly higher in group B than group A; despite being within the normal range, it was relatively deficient on the basis of the age of the participants in the control group.

Conclusion
Testosterone deficiency is a prevalent condition in CKD that starts at an earlier age than the normal population. It is an evident independent cause of anemia in EPO-naive CKD patients and is a possible cause of resistance of ESAs in CKD patients; still, the most important causes of anemia in CKD are EPO and iron deficiency.

Keywords:
anemia, chronic kidney disease (CKD), erythropoiesis stimulating agents (ESAs), testosterone deficiency

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Introduction

Chronic kidney disease (CKD) is a disease that is prevalent worldwide. Uremic manifestations in patients with CKD stage 5 are believed to be primarily secondary to the accumulation of uremic toxins. It is indicated by the clinical onset of the following symptoms: nausea, vomiting, fatigue, anorexia, weight loss, muscle cramps, pruritus, mental status changes, visual disturbances, and increased thirst. Patients may report nonspecific symptoms, which become chronic and progressive over time because of the gradual onset of the disease. The diagnosis of uremia may be difficult in young children because of the nonspecificity of clinical symptoms [1]. Metabolic abnormalities such as anemia, acidemia, and electrolyte abnormalities are prominent. Anemia is very common in patients with CKD. It is associated with reduced quality of life and increased cardiovascular disease, hospitalization, cognitive impairment, and mortality [2]. Although the pathogenesis is multifactorial, it mainly results from an erythropoietic hypoproliferative state because of the relative insufficiency in erythropoietin (EPO) production by the failing kidney [3]. The use of EPO has revolutionized the management of renal anemia by improving the patient's debilitating symptoms and freeing them from dependance on blood transfusions, with its associated complications [4]. Nevertheless, ~5–10% of patients show an inadequate response to EPO doses [5]. Identification of the causes of EPO

resistance or unresponsiveness can optimize the management of anemia and improve the financial costs and safety of EPO therapy [5]. The European Best Practice Guidelines for the management of anemia in patients with CKD propose that the lower limit of normal for hemoglobin (Hb) be 11.5 g/dl in women, 13.5 g/dl in men at or younger than 70 years of age, and 12.0 g/dl in men older than 70 years of age [6].

The National Kidney Foundation's and Kidney Dialysis Outcomes Quality Initiative (K/DOQUI) recommends a workup for anemia in patients with CKD if the Hb level is less than 11.0 g/dl (hematocrit<33%) in premenopausal women and prepubertal patients and when the Hb is less than 12.0 g/dl (hematocrit <37%) in adult men and postmenopausal women [7]. African Americans and patients with diabetes have even higher rates of anemia at each stage of kidney disease [8]. Anemia of chronic illness and anemia of CKD both fall under the category of decreased red blood cell (RBC) production, which is normochromic, normocytic anemia [9]. Factors likely contributing toward anemia in CKD include (differential diagnosis) EPO deficiency, iron deficiency, blood loss, shortened red cell life span, vitamin (D, B_{12}) and folic acid deficiencies, the uremic milieu, inflammation, bone marrow fibrosis (hyperparathyroidism), other reasons – for example, pure red cell aplasia, bone marrow infiltration, aluminum toxicity, malignancy, hemolysis, and hypothyroidism [10–12]. The most important causes of anemia in CKD are iron and EPO [10].

Erythropoietin deficiency

EPO is a glycoprotein with the main function of stimulating the proliferation and differentiation of erythroid precursors in the bone marrow. It is produced mainly in the kidneys, although several other tissues produce lesser amounts, mainly the liver (which is considered the main extrarenal site). EPO transcription and release of EPO into the blood stream are both induced by hypoxic conditions. The EPO gene has a hypoxia-responsive element, a sequence that induces expression when bound to hypoxia-inducible factor-1, a transcription factor. There are two types of EPO receptors: high-affinity, high-specificity receptors, expressed predominantly on hematopoietic cells, and low-affinity, low-specificity receptors expressed more broadly on nonhematopoietic cells. The high-affinity receptors will respond to lower levels of EPO, whereas the low-affinity receptors require a much higher level to induce a response. The EPO receptors expressed on nonhematopoietic cells are functional, although their role has not been identified [13]. EPO deficiency is considered the most important cause of anemia in CKD. It is postulated that the specialized peritubular cells that produce EPO are partially or completely depleted or injured as renal disease progresses so that EPO production is inappropriately low relative to the degree of anemia.

Erythropoietin-stimulating agents resistance

Erythropoietin-stimulating agent (ESAs) should be administered to achieve and maintain a target Hb concentration of 11.0–12.0 g/dl. There is currently no evidence that increasing the Hb to normal levels offers any significant clinical advantage over this target goal [14]. Two ESAs are used: epoietin alfa and darbepoetin alfa. The two agents available for the treatment of anemia in CKD are recombinant epoietin alfa (Epogen and Procrit) and darbepoetin alfa (Aranesp); both are effective and safe [14]. They can be administered intravenously or subcutaneously. ESAs resistance is defined by the KDIGO guidelines as no increase in the Hb concentration from baseline after the first month of ESAs treatment on appropriate weight-based dosing or requiring two increases in ESAs doses up to 50% beyond the previous maintenance dose, after a stable treatment with ESAs, to maintain a stable Hb concentration [15]. The most common cause of ESAs hyporesponsiveness is iron deficiency, whether related to absolute iron deficiency or inability to access iron stores because of a chronic inflammatory state. Another fairly common cause of ESAs hyporesponsiveness in CKD patients is severe hyperparathyroidism. As always, other causes of anemia must be ruled out, including other vitamin deficiencies, occult bleeding, hemoglobinopathies, bone marrow disorders, hypothyroidism, chronic infection or inflammation, statin, aluminum toxicity, malignancy, insulin resistance, and high altitude. Given the significant prevalence of ESAs hyporesponsiveness, its impact on morbidity and mortality, as well as increased costs related to higher doses of ESAs, hospitalization, and transfusions, ESAs hyporesponsiveness is an important issue that needs to be addressed [16–18].

Forms of iron deficiency

Iron deficiency is also common in patients with CKD. The iron deficiency may be as follows:

(i) Absolute iron deficiency, often because of poor dietary intake or sometimes occult bleeding, and
(ii) Functional iron deficiency, when there is an imbalance between the iron requirements of the erythroid marrow and the transferrin-bound circulating iron supply [19].

Absolute iron deficiency

Absolute iron deficiency is defined as transferring saturation (TSAT) less than 20% and a serum ferritin

concentration less than 100 ng/ml [14,20]. However, it is useful to divide iron deficiency into two parts:

(a) Total body iron deficiency, as reflected by a low ferritin, and
(b) Accessible iron deficiency, as reflected by a low TSAT.

In inflammatory states, because of the actions of hepcidin, there is often an accessible iron deficiency in the presence of a normal or increased, often markedly increased, ferritin. In patients on hemodialysis, the prevalence of iron deficiency is still higher, both because of an increased severity of chronic inflammation and also because of chronic blood loss in the hemodialysis circuit [21].

Functional iron deficiency

Patients who do not respond to ESA therapy despite having adequate iron stores are considered to have a functional or a relative iron deficiency. It is unique to the population of patients who are being treated with these ESAs because their supraphysiologic rate of RBC production outstrips the ability of transferrin-bound circulating iron to provide an adequate substrate for Hb synthesis. In these patients, who have a functional or relative iron deficiency, the TSAT < 20% as bone marrow strips iron off the circulating transferrin faster than the transferrin can replenish it with iron released from stores. The serum ferritin, which reflects iron stores, may be normal or increased. This is a problem of supply and demand, not total body iron deficiency [22].

Testosterone deficiency and anemia of chronic kidney disease

Hypogonadism or testosterone deficiency is a prevalent condition in men with CKD [23]. The association between androgens and erythropoiesis has been known for more than seven decades, and one common effect of testosterone therapy in nonrenal populations is an increase in hemoglobin levels [24]. Although testosterone deficiency is present in up to 60% of men with CKD, hypogonadism has so far received relatively little attention in practice [25].

Mechanism and causes of hypogonadism

Although the exact reason(s) for a reduction in free testosterone levels in CKD are not fully evident, it could be because of prolactin retention and subsequent inhibition of gonadotropins [25].

Testosterone and erythropoiesis

The exact mechanism(s) by which testosterone stimulates erythropoiesis is, however, not yet evident. Although androgens have been reported to have myelostimulating effects by inducing the production of hematopoietic growth factors in bone marrow stromal cells [26], it has been speculated that testosterone may also influence iron bioavailability [27]. Before the introduction of recombinant human EPO into clinical practice, androgens were sometimes used to treat anemia in dialysis patients [28,29]. Androgens have also been suggested as adjuvants to ESA in the treatment of CKD patients with anemia, but the risk of side effects has precluded its use in large numbers of dialysis patients [30].

Aim

The aim of this study was to evaluate serum testosterone level in patients on MHD and correlate its level with anemia and response to ESAs therapy.

Patients and methods

This study included 40 male patients from dialysis units, where they were divided into two groups: group A, group taking ESAs, and group B ESAs, group not taking (EPO-naive group). Another 20 men were included as group C (control group).

Methods

All groups were subjected to a full assessment of history, full clinical examination, and laboratory investigations to determine complete blood count (CBC), reticulocyte hemoglobin (CHr), erythrocyte sedimentation rate (ESR), parathormone hormone (PTH), high sensitivity C reactive protein (hsCRP), EPO level, free serum testosterone, serum urea and creatinine (before mid week session), HBVsAg, HCV Ab, and HIV Ab to exclude the possible causes of anemia.

Results

Demographic data

Table 1 shows the age distribution among the different groups, where group A included 20 patients taking ESAs. Five patients were between 25 and 35 years old, another five patients were between 25and 40 years old, and 10 patients were between 45 and 55 years old. The mean age of the group was 45.45 ± 9.15 years. Group B included 20 patients not taking ESAs. They had the same age distribution as group A, but the mean age of the group was 44.10 ± 9.28 years. Group C included 20 normal participants of the same age distribution as groups A and B, but the man age of the group was 34.60 ± 9.49 years. There was no significant difference between the two groups and the control group in age.

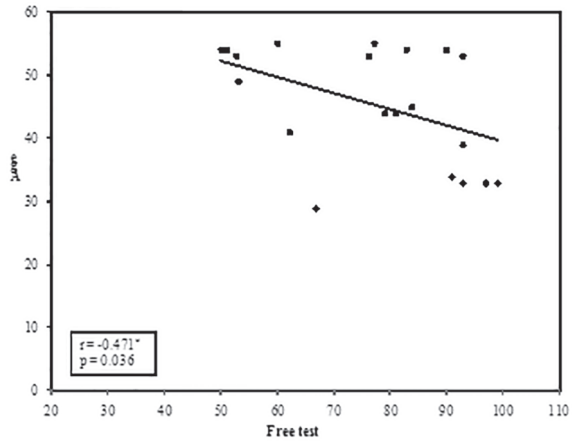
Statistical correlation

This study showed a negative correlation between age and Hb and also between age and EPO in both patient groups (groups A and B) as well as a positive correlation between Hb and EPO in the same groups. This study showed a statistically significant negative correlation between free testosterone and age in both patient groups (A and B) (Figs. 1–3) as well as a statistically significant positive correlation between free testosterone and Hb in all sample groups (A+B+C) (Fig. 4). Comparison of free testosterone levels in both patient groups showed that it was almost equal, with a significant decrease with age. This study showed a statistically significant positive correlation between free testosterone and EPO in all sample groups (A + B + C) (Fig. 5).

Discussion

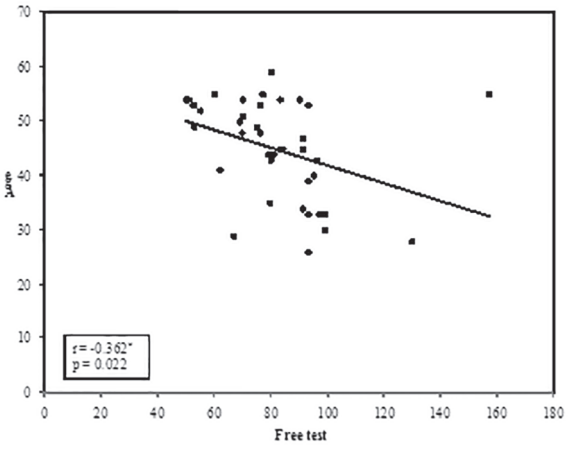
This study showed a negative correlation between age and Hb and also between age and EPO in both patient groups (groups A and B) as well as a positive correlation between Hb and EPO in the same groups. This is because of the pathological decrease in serum EPO level owing to failed EPO synthesis by failed kidney (which represents 90% of the total EPO in the body) [31]. Many studies such as, Hawkins *et al*. [32], Salive *et al*. [33], and Yip *et al*. [34] reported the same results. Jorien *et al*. [22], who studied the relation between EPO, Hb, and renal function in the oldest old, population above 85 years old, it concluded that only when creatinine clearance is lower than 30 ml/min is a relatively low EPO response found. Other studies were carried out to prove that there is no decrease in EPO with age such as Musso *et al*. [35], who concluded that normal senescence does not alter plasma EPO levels, even during advanced aging. Neither of these studies was carried out on the stage 5 CKD on MHD and this study was carried out in a younger age group, and not in the elderly. This study showed a statistically significant negative correlation between free testosterone and age in both patient groups (A and B) as well as a statistically significant

Figure 1



Correlation between age and free testosterone in taker (group A).

Figure 2



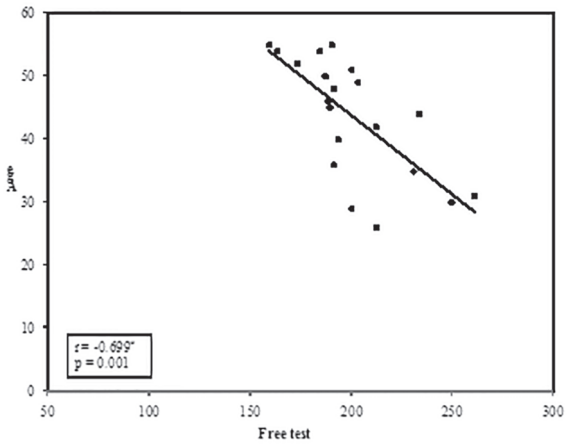
Correlation between age and free testosterone in all patients (group A + B).

Table 1 Comparison between the studied groups according to demographic data (age and sex)

Demographic data	Taker (group A) (*n* = 20)		Nontaker (group B) (*n* = 20)		Control (group C) (*n* = 20)		Test of significance	*P*
	No.	%	No.	%	No.	%		
Sex								
Male	20	100.0	20	100.0	20	100.0	–	–
Female	0	0.0	0	0.0	0	0.0		
Age (years)								
25–35	5	25.0	5	25.0	5	25.0	χ^2 = 0.0	1.000
>35–45	5	25.0	5	25.0	5	25.0		
>45	10	50.0	10	50.0	10	50.0		
Minimum–maximum	29–55		26–59		26–55		*F* = 0.211	0.810
Mean ± SD	45.45 ± 9.15		44.10 ± 9.28		34.60 ± 9.49			
Median	47.0		46.0		45.50			

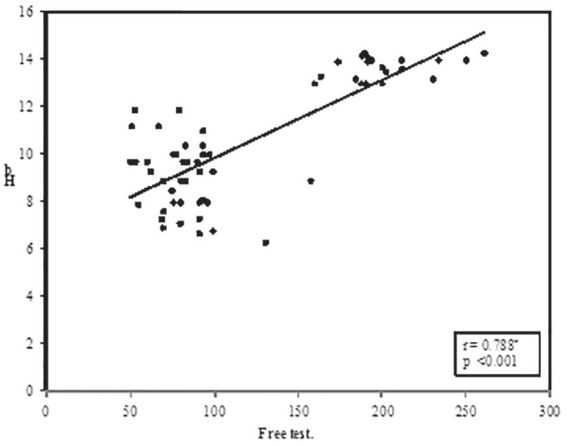
F, *F*-test (ANOVA), χ^2: Chi square test.

Figure 3



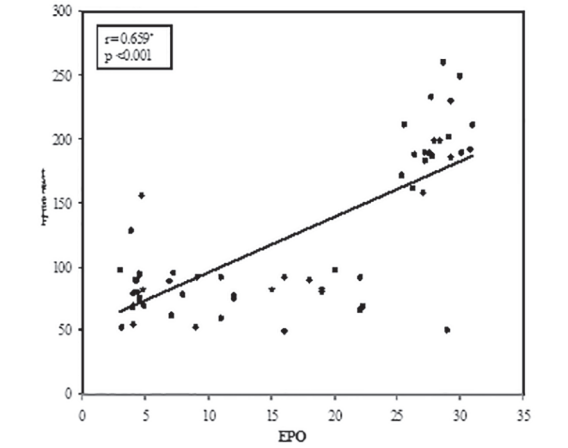
Correlation between age and free testosterone in the control group (group C).

Figure 4



Correlation between hemoglobin (Hb) and free testosterone in the total sample (group A + B + C).

Figure 5



Correlation between free testosterone and erythropoietin (EPO) in the total sample (group A + B + C).

positive correlation between free testosterone and Hb in all sample groups (A + B + C). Comparison of free testosterone level in both patients groups showed that it was almost equal, with a significant decrease with age. This study showed a statistically significant positive correlation between free testosterone and EPO in all sample groups (A + B + C). This decrease in testosterone level is because of primary hypergonadotrophic hypogonadism owing to senescence that becomes evident after 60 years of age [36], which explains the negative correlation with age. Testosterone hormone stimulate Hb synthesis by facilitating intestinal iron resorption and enhancing iron incorporation in RBCs, and also by stimulating erythropoiesis by stimulating erythroid colony formation and enhancing differentiation into EPO receptors rather than leukocytes [37], which explains the positive correlation with Hb and EPO levels. Carrero *et al.* [23] showed that high levels of ESAs are correlated negatively to testosterone level. This could be because of the counter-regulatory effect on the androgen mechanism by which it stimulates erythropoiesis, as shown in Lacomb *et al.* [38], where there is a negative feedback by red cell mass on EPO production that is facilitated through the renal oxygen sensor, which has a different threshold, thus explaining the differences in the dose–response relationship of EPO with androgen exposure. Kalmanti *et al.* [39] reported on the same correlation between testosterone and EPO level as this study through bone marrow biopsies in patients on dialysis and receiving androgen therapy. Also, many other studies such as Ramirez *et al.* [40], Kokot *et al.* [41], and Schaefer *et al.* [42] reported the same conclusion between testosterone and EPO level. Svartberg *et al.* [43] as well as many studies confirmed an age-related decrease in both total and free testosterone. This study showed that 75% of the participants in group A were anemic, whereas 100% of the participants in group B were anemic, with a higher degree of anemia. The testosterone level was slightly higher in group B than group A; despite being within the normal range, it was relatively deficient on the basis of the age of the participants in the control group.

Conclusion

Testosterone deficiency is a prevalent condition in CKD that starts at an earlier age than in the normal population. It is an evident independent cause of anemia in EPO-naive CKD patients and it is a possible cause of resistance of ESAs in CKD patients. Still, the most important causes of anemia in CKD are EPO and iron deficiency.

Acknowledgements

Conflicts of interest

None declared.

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