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Circulating Fibroblast Growth Factor-23 Level and Paraoxonase-1 Lactonase Activity in Chronic Hemodialysis Patients: Their Impact on the Incidence of Native AV Fistula Thrombosis

Abstract

Purpose: Thrombosis of native arteriovenous (AV) fistula is an important cause of complications in hemodialysis (HD) patients. The purpose of this study was to investigate the usefulness of measuring circulating fibroblast growth factor-23 (FGF-23) level and paraoxonase-1 (PON1) lactonase activity as potential predictors of native AV fistula thrombosis in chronic HD patients.

Methods: This study included 83 HD patients (48 with thrombosed and 35 with non-thrombosed native AV fistulas) and 38 healthy volunteers. Serum FGF-23 level was measured using the ELISA technique, while serum PON1 lactonase activity was measured spectrophotometrically using gamma-thiobutylolactone as a substrate.

Results: FGF-23 was significantly increased while PON1 lactonase was markedly decreased in both thrombosed and non-thrombosed HD patients compared with controls ($P < 0.001$). FGF-23 was elevated whereas PON1 lactonase was decreased in HD patients with thrombosed native AV fistulas compared with HD patients with non-thrombosed native AV fistulas ($P = 0.001$ and 0.002 , respectively). A significant negative correlation was found between FGF-23 and PON1 lactonase in HD patients with thrombosed native AV fistulas ($r = -0.342$, $P = 0.017$).

Conclusions: This study shows a potential value of FGF-23 and PON1 lactonase as predictors of native AV fistula thrombosis in HD patients.

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Loss of vascular access is known to be an important cause of morbidity and mortality in hemodialysis (HD) patients [1,2]. Permanent vascular access in HD patients is provided through a central venous catheter, arteriovenous (AV) graft or native AV fistula [3]. Arteriovenous thrombosis accounts for 80-85% of vascular access loss, and vascular stenosis developing in venous segment of native AV fistula or AV graft is the major predisposing factor for thrombosis, being responsible for 80% of the cases [2]; therefore, it is extremely important to identify the predictors of native AV fistula thrombosis in HD patients.

There are 18 mammalian fibroblast growth factors (FGF-1 to FGF-10 and FGF-16 to FGF-23) that are grouped into six subfamilies based on differences in sequence homology and phylogeny [4]. FGF-23 is a 251 amino acid protein (MW 26 kDa) synthesized and secreted by bone cells, mainly osteoblasts [5]. FGF-23 exerts its biological effects through activation of FGF receptors (FGFRs) in a Klotho-dependent manner. In the presence of Klotho, FGF-23 induces the phosphorylation and subsequent activation of the extracellular signal-regulated kinase in various types of cells [6]. Klotho mRNA expression is detected in occlusive coronary thrombi obtained from patients with acute coronary syndrome [7].

In patients with chronic kidney disease (CKD), FGF-23 levels rise in parallel with renal function decline [8]. Serum FGF-23 level in HD patients has been reported to be significantly higher than in healthy subjects [9,10]. FGF-23 is a central regulator of phosphate homeostasis and calcitriol blood levels. FGF-23 enhances renal phosphate excretion in order to maintain serum phosphate levels within the normal range. FGF-23 mediates its phosphaturic effect by inhibiting the Na/Pi IIa cotransporter in renal proximal tubules [11]. Moreover, FGF-23 inhibits gastrointestinal phosphate absorption by reducing intestinal Na/Pi IIb cotransporter activity in a vitamin D receptor-dependent manner [12]. In mice, injection of recombinant FGF-23 reduces calcitriol levels by decreasing renal expression of 1α -hydroxylase, the rate-limiting step in the generation of calcitriol, and increasing the expression of 24-hydroxylase, which controls calcitriol degradation [13]. Calcitriol deficiency induces parathyroid hormone (PTH) secretion and thus secondary hyperparathyroidism (HPT) [11]. Increased FGF-23 levels were found to predict left ventricular hypertrophy in HD patients [14]. Data from clinical trials suggest that high FGF-23 level is associated with endothelial dysfunction in subjects with clinically overt renal failure [15]. High serum FGF-23 level has been shown to be associated with total body atherosclerosis in the community and this association was stronger in individuals with diminished renal function [16].

The effect of FGF-23 level on the incidence of native AV fistula thrombosis in HD patients is unknown.

The human paraoxonase (PON) gene family consists of three members: *PON1*, *PON2* and *PON3*. *PON1* is synthesized in the liver and secreted into the blood, where it associates exclusively with a specific HDL sub-species that also contains apolipoprotein (apo) A-I and clusterin. *PON1* is a glycoprotein of 354 amino acids and an approximate molecular mass of 43 kDa. On ultracentrifugation, the majority of *PON1* (and HDL antioxidant activity) resides on the small dense HDL3 subfraction [17,18]. *PON1* possesses paraoxonase, arylesterase and lactonase activities; *PON2* and *PON3* have lactonase activity but practically no paraoxonase or arylesterase activity. The levels of serum *PON3* are very low compared with *PON1* levels and *PON2* is not present in serum at all [17]; therefore, the lactonase assay predominantly reflects the lactonase activity of *PON1*.

The thioester homocysteine (Hcy)-thiolactone, the product of an error-editing reaction in protein biosynthesis, is a reactive metabolite that causes protein *N*-homocysteinylolation that affects protein structure and function (e.g., enzymes lose their activity, *N*-Hcy-fibrinogen acquires prothrombotic properties and *N*-Hcy-proteins become autoimmunogenic). *PON1* lactonase activity detoxifies Hcy-thiolactone in human blood and could be a potential contributing factor in the prevention of atherosclerosis [19,20]. *In vitro* studies showed that lactonase activity of *PON1* prevented Hcy-thiolactone and *N*-Hcy-protein accumulation in cultures of human endothelial cells [21]. It was reported that *PON1* lactonase activity, stimulated by reconstituted HDL containing apo A-I, mediated the protection of LDL from oxidation and stimulated the HDL-mediated cholesterol efflux from macrophages [22]. Previous studies have demonstrated decreased *PON1* lactonase activity in HD patients compared with healthy subjects [23–25], moreover; HD patients were found to be susceptible to lipid peroxidation, protein homocysteinylolation and, hence, atherosclerosis [23,24]. *PON1* lactonase activity was significantly lowered in patients with coronary artery disease (CAD) than in controls [26,27]. Serum levels of total Hcy and oxidized LDL increased significantly in CAD patients and were associated with a significant decrease in both the levels of HDL cholesterol and the *PON1* lactonase activity. In CAD patients, the *PON1* lactonase activity was negatively associated with total Hcy and high-sensitivity C-reactive protein (CRP) levels [26]. The possible association between *PON1* lactonase activity and occurrence of AV fistula thrombosis in HD patients remains unexplored.

The aim of our study was to evaluate the possibility of using serum FGF-23 level and PON1 lactonase activity as reliable predictors of native AV fistula thrombosis in HD patients. FGF-23 level and PON1 lactonase activity were compared in HD patients with different numbers of native AV fistula thrombosis during the previous 3 years. Furthermore, we assessed whether there was an association between these two parameters in HD patients with thrombosed and non-thrombosed AV fistulas.

Materials and methods

Subjects

Eighty-three chronic renal failure patients (mean age = 56.18 years $\pm$ 3.98 SD, 45 men and 38 women, body mass index (BMI) = 26.02 kg/m² $\pm$ 2.10 SD, 24 smokers and 59 non-smokers) on maintenance HD were enrolled in this study. Patients were selected from the Nephrology Department, Faculty of Medicine, Ain Shams University Hospitals, Cairo, Egypt. The patients were routinely hemodialyzed three times per week in sessions lasting 4 h, using Fresenius polysulfone dialyzer membrane. The dialysate flow rate was 500 mL/min and blood flow rates ranged from 200–250 mL/min. Each patient was treated with a single dose of 70 IU/kg enoxaparin just prior to the start of HD. Patients with previously experienced cerebrovascular disease, history of myocardial infarction, diffuse atherosclerosis, peripheral arterial disease, active infection, autoimmune disease, diabetes mellitus, liver dysfunction or malignancies were excluded from the study. None of the patients received antibiotics, immunosuppressive treatment, lipid-lowering agents or non-steroidal anti-inflammatory drugs at the time of the study. A total of 44 patients were on treatment with erythropoietin (mean weekly dose = 5617.0 U $\pm$ 690.28 SD). Fifty-one patients were being treated with antihypertensive drugs (angiotensin-converting enzyme inhibitors, β -blockers or calcium channel blockers). The causes of renal failure included glomerulonephritis in 29, nephrosclerosis in 24, obstructive nephropathy in 21, polycystic kidney disease in four and unknown in five cases. All patients had native AV fistulas. AV fistula thrombosis was defined as the lack of blood flow, assessed by palpation and auscultation. The presence of thrombus was confirmed by duplex ultrasound. The number of AV fistula thrombosis was recorded for each patient during the previous 3 years. Thirty-five patients had no episodes of AV fistula thrombosis, 18 patients had one episode of AV fistula thrombosis, 16 patients had two episodes of AV fistula thrombosis and 14 patients had more than two episodes of AV fistula thrombosis.

A group of 38 healthy volunteers matched for age, gender, BMI and smoking status (mean age = 53.64 years $\pm$ 4.39 SD, 20 men and 18 women, BMI = 24.91 kg/m² $\pm$ 2.19 SD, 11 smokers and 27 non-smokers) was also included in this study. The study was performed in accordance with Declaration of Helsinki, and was approved by the Research Ethics Committee of Ain Shams University, Faculty of Medicine, Cairo, Egypt. An informed consent was obtained from all patients and controls.

Biochemical measurements

Blood samples of HD patients were collected from the AV fistulas immediately before the beginning of a routine 4-hour HD session. With respect to controls, blood samples were drawn from the antecubital vein. Serum samples were prepared, aliquoted and stored at -70°C until analyses. FGF-23 (C-term) was measured by ELISA technique using a quantitative kit manufactured by Immutopics, Inc., San Clemente, CA, USA. Briefly, two affinity purified goat polyclonal antibodies were selected to detect epitopes within the carboxyl-terminal portion of FGF-23. One antibody was biotinylated for capture and the other antibody was conjugated with the enzyme horseradish peroxidase (HRP) for detection. A sample containing human FGF-23 was incubated simultaneously with the biotinylated capture antibody and the HRP conjugated antibody in a streptavidin coated microtiter well. FGF-23 contained in the sample was immunologically bound by the capture antibody and the detection antibody to form a “sandwich” complex. At the end of this incubation period, the well was washed to remove any unbound antibody and other components. The enzyme bound to the well was incubated with a substrate solution (tetramethylbenzidine with hydrogen peroxide) in a timed reaction and then measured using ELISA plate reader (Labsystems Multiskan[®] plus, Helsinki, Finland). Intra-assay coefficient of variation (CV) ranged from 1.7% to 2.9%, while inter-assay CV ranged from 3.1% to 5.2% for FGF-23. The calculated analytical recovery ranged from 92.8% to 103.7% for FGF-23. PON1 lactonase activity was measured by a commercially available assay kit (Alfresa Auto HTLase; Alfresa Pharma Corporation, Osaka, Japan). This kit utilized gamma-thiobutyrolactone as substrate and Elman's procedure to monitor the accumulation of free sulfhydryl groups via coupling with 5,5'-dithiobis(2-nitrobenzoic acid). Intra-assay CV ranged from 4.1% to 5.6%, whereas inter-assay CV ranged from 3.9% to 5.8% for paraoxonase-1.

Statistical analysis

The Kruskal–Wallis and Mann–Whitney *U* non-parametric tests were used for the statistical comparison of the variables between the various groups. The non-parametric Spearman's correlation analysis was utilized to study the association between the different variables. The independent samples *t*-test $\pm$ SD with 95% confidence interval (CI) was used to compare the baseline characteristics of the HD patients. Values of *P* lower than 0.05 were considered statistically significant. These analyses were performed using the Statistical Package for the Social Sciences (SPSS software, version 20.0, Armonk, NY, USA).

Results

Demographic characteristics of chronic HD patients are shown in Table 1. No significant difference was found between the HD patients with thrombosed and non-thrombosed native AV fistulas in terms of age, gender, BMI, HD duration,

smoking status, erythropoietin therapy, blood pressure and etiology of CKD.

Table 2 reveals a significant increase in the median levels of FGF-23 and PON1 lactonase among controls, all HD patients, HD patients with thrombosed AV fistulas and HD patients with non-thrombosed AV fistulas (chi-square = 103.151, 102.077, respectively; *P* < 0.001 for the two parameters). The median level of FGF-23 was significantly elevated, whereas the median level of PON1 lactonase was significantly decreased in HD patients compared with healthy subjects (*Z* = −8.807, −8.559, respectively; *P* < 0.001 for the two parameters). Furthermore, the median level of FGF-23 was significantly elevated (*Z* = −3.463, *P* = 0.001), while the median level of PON1 lactonase was significantly decreased (*Z* = −3.095, *P* = 0.002) in HD patients with thrombosed AV fistulas compared with HD patients with non-thrombosed AV fistulas.

We found a statistically significant difference in the median levels of FGF-23 (chi-square = 11.938, *P* = 0.003) and

TABLE 1. Demographic characteristics of chronic HD patients with thrombosed and non-thrombosed native AV fistulas using independent samples *t*-test

Variables	HD patients with thrombosed AV fistulas (<i>n</i> = 48)	HD patients with non-thrombosed AV fistulas (<i>n</i> = 35)	<i>P</i> value
<i>Age</i> Mean (years) $\pm$ SD	57.21 $\pm$ 3.98	55.14 $\pm$ 4.39	0.546
<i>Gender</i> Male/female, <i>n</i>	27/21	18/17	0.669
<i>BMI</i> Mean (kg/m ²) $\pm$ SD	26.54 $\pm$ 2.14	25.49 $\pm$ 2.07	0.126
<i>HD duration</i> Mean (years) $\pm$ SD	9.02 $\pm$ 5.17	8.73 $\pm$ 4.93	0.647
<i>Smoking status</i> Smokers/none-smokers, <i>n</i>	16/32	8/27	0.296
<i>Erythropoietin therapy</i> Treated/non-treated, <i>n</i>	26/22	18/17	0.808
<i>Blood pressure</i> Hypertensive/normotensive, <i>n</i>	30/18	21/14	0.82
<i>Etiology of CKD</i>			
Glomerulonephritis, <i>n</i>	17	12	0.916
Nephrosclerosis, <i>n</i>	14	10	0.954
Obstructive nephropathy, <i>n</i>	12	9	0.942
Polycystic kidney disease, <i>n</i>	2	2	0.755
Unknown, <i>n</i>	3	2	0.92

TABLE 2. FGF-23 level and PON1 lactonase activity in serum of healthy subjects and chronic HD patients with thrombosed and non-thrombosed native AV fistulas using non-parametric Kruskal–Wallis test

Groups	Normal (<i>n</i> = 38)	All HD Patients (<i>n</i> = 83)	HD patients with thrombosed AV fistulas (<i>n</i> = 48)	HD patients with non-thrombosed AV fistulas (<i>n</i> = 35)
<i>FGF-23 level (RU/mL)</i>				
Mean rank	19.5	121.48	139.72	96.57
Range	31.6–84.3	791.4–7693.6	927.5–7693.6	791.4–2623.1
Median	53.1	1903.2	2300.8	1397.5
chi-square = 103.151, <i>P</i> < 0.001*				
<i>PON1 lactonase activity (U/L)</i>				
Mean rank	183.16	84.04	62.7	113.3
Range	175.1–347.3	71.6–202.5	71.6–187.2	98.2–202.5
Median	239.2	133.7	107.5	145.6
chi-square = 102.077, <i>P</i> < 0.001*				

Comparison by Mann–Whitney *U* test.

FGF-23 was significantly increased, while PON1 lactonase was significantly decreased in HD patients compared with controls (*Z* = −8.807, and −8.559, respectively; *P* < 0.001* for the two parameters).

FGF-23 was significantly increased, while PON1 lactonase was significantly decreased in HD patients with thrombosed AV fistulas compared with patients with non-thrombosed AV fistulas (*Z* = −3.463, *P* = 0.001 for FGF-23; *Z* = −3.095, *P* = 0.002 for PON1 lactonase).

**P* value is significant.

TABLE 3. FGF-23 level and PON1 lactonase activity in serum of chronic HD patients with thrombosed native AV fistulas using non-parametric Kruskal–Wallis test

Number of AV fistula thrombosis in previous 3 years	One episode (<i>n</i> = 18)	Two episodes (<i>n</i> = 16)	More than two episodes (<i>n</i> = 14)
<i>FGF-23 level (RU/mL)</i>			
Mean rank	17.08	23.69	34.96
Range	927.5–3612.7	1203.2–4731.9	1516.1–7693.6
Median	1688.3	2101.6	3703.2
Chi-square = 11.938, <i>P</i> = 0.003*			
<i>PON1 lactonase activity (U/L)</i>			
Mean rank	31.28	24.01	16.36
Range	84.7–187.2	76.5–167.1	71.6–158.9
Median	131.4	99.1	84.3
Chi-square = 8.984, <i>P</i> = 0.011*			

Comparison by Mann–Whitney *U* test

FGF-23 was significantly increased, while PON1 lactonase was significantly decreased in HD patients with more than two episodes of AV fistula thrombosis compared with patients with one episode (*Z* = −3.382, *P* = 0.001 for FGF-23; *Z* = −2.793, *P* = 0.005 for PON1 lactonase). PON1 lactonase activity was significantly decreased in HD patients with two episodes of AV fistula thrombosis compared with patients with one episode (*Z* = −2.089, *P* = 0.037).

**P* value is significant.

TABLE 4. Associations between serum FGF-23 level and PON1 lactonase activity in chronic HD patients with thrombosed and non-thrombosed native AV fistulas using non-parametric Spearman's correlation analysis

Groups	Correlation between <i>FGF-23</i> levels and <i>PON1</i> lactonase activity
All HD patients ($n=83$)	$r = -0.252, P = 0.021^*$
HD patients with thrombosed AV fistulas ($n=48$)	$r = -0.342, P = 0.017^*$
HD patients with one episode of AV fistula thrombosis ($n=18$)	$r = -0.518, P = 0.028^*$
HD patients with two episodes of AV fistula thrombosis ($n=16$)	$r = -0.558, P = 0.025^*$
HD patients with more than two episodes of AV fistula thrombosis ($n=14$)	$r = -0.770, P = 0.001^*$
HD patients with non-thrombosed AV fistulas ($n=35$)	$r = -0.352, P = 0.038^*$

r values are Spearman's correlation coefficients.

* P value is significant.

PON1 lactonase (chi-square = 8.984, $P = 0.011$) among HD patients with one episode, two episodes and more than two episodes of AV fistula thrombosis. Moreover, the median level of FGF-23 was significantly increased ($Z = -3.382, P = 0.001$), while the median level of PON1 lactonase was significantly decreased ($Z = -2.793, P = 0.005$) in HD patients with more than two episodes of AV fistula thrombosis compared with patients with one episode. The median level of PON1 lactonase was significantly decreased in HD patients with two episodes of AV fistula thrombosis compared with patients with one episode ($Z = -2.089, P = 0.037$) (Table 3).

Table 4 showed a significant negative correlation between FGF-23 and PON1 lactonase in all HD patients ($r = -0.252, P = 0.021$), in HD patients with thrombosed ($r = -0.342, P = 0.017$) and non-thrombosed ($r = -0.352, P = 0.038$) AV fistulas. FGF-23 was negatively correlated with PON1 lactonase in HD patients with one ($r = -0.518, P = 0.028$), two ($r = -0.558, P = 0.025$), and more than two episodes ($r = -0.770, P = 0.001$) of AV fistula thrombosis.

Discussion

A major factor that facilitates AV fistula failure in HD patients is thrombosis, which is due to intimal hyperplasia developing in the venous segment of AV fistula [2]. Good vascular access is vital to attain effective dialysis treatment, keep patients' quality of life at a maximum level and prevent unnecessary loss of time and cost increases. In the present study, we showed that elevated concentrations of serum FGF-23 and low activity of serum PON1 lactonase might be reliable predictors for the incidence of native AV fistula thrombosis in chronic HD patients.

In accordance with previous studies [9,10], we detected a marked increase in serum FGF-23 levels in HD patients compared with healthy controls. Results of the NephroTest study revealed that declining renal function was associated with a number of CKD-related metabolic complications, including HPT, anemia, acidosis, hyperkalemia and hyperphosphatemia [28]. PTH may stimulate FGF-23 secretion by osteoblasts, as FGF-23 levels were found to be increased in rodents with primary HPT, which may be reversed by parathyroidectomy [29]. Transgenic mice overexpressing human FGF-23 exhibited hypophosphatemia with increased phosphate wasting, low serum calcitriol and rachitic bone [30]. In contrast, the FGF-23 null mice displayed significantly high serum phosphate levels with increased renal phosphate reabsorption, and an elevation in serum calcitriol level [31]. These observations confirm the indispensable role of FGF-23 in the physiological regulation of phosphate and calcitriol. Administration of neutralizing antibodies that target FGF-23 normalized phosphate and calcitriol concentrations in the serum of mice with hypophosphatemic rickets [32]. In HD patients, increased serum FGF-23 level was associated with mortality independently of serum phosphate levels [9,10].

In the present study, the increase in FGF-23 was most pronounced in HD patients with thrombosed AV fistulas compared with patients with non-thrombosed AV fistulas. Based on the current data, it could appear that the serum FGF-23 level is a potential new predictor of AV shunt thrombosis in HD patients. Higher FGF-23 was associated with both impaired vasoreactivity in subjects with normal renal function, and increased arterial stiffness in subjects with impaired renal function [15]. FGF-23 and carotid artery intima-media thicknesses (as a marker of atherosclerosis) were

found to be significantly higher in HD [33] patients than controls. Higher FGF-23 level was associated with greater likelihood and burden of carotid atherosclerosis [34]. Increased serum FGF-23 levels were associated with aortic [35], peripheral vascular [36] and coronary artery [37] calcifications in HD patients. Previously, it was shown that anti-FGF-23 monoclonal antibodies suppressed PTH and caused an elevation in the calcitriol and phosphate levels in CKD-rat model [38]. The favourable changes in PTH and calcitriol levels support the use of FGF-23 antibodies as a potential therapeutic strategy in CKD; however, the worsening hyperphosphatemia indicates that the increased FGF-23 level might be therapeutic. Much knowledge and wisdom are needed before we can move towards using FGF-23 as a possible therapeutic target in CKD patients.

We found that serum PON1 lactonase activity was significantly decreased in HD patients compared with normal volunteers. Similarly, serum PON1 activities, including lactonase, were reported to be lower in end-stage renal disease patients undergoing HD compared with healthy controls [23–25,39,40]. A lower serum PON1 lactonase activity would be coupled with delayed catabolism of oxidized phospholipids in LDL and oxidized macrophages, and with greater protein homocysteinylation, accelerating atherogenesis [23,24]. Previous studies showed that HD significantly restored PON1 activities. Moreover, an inverse association between PON1 activities recovery after HD was found with creatinine, advanced glycation end products and acrolein, suggesting that uremic toxins may play a mechanistic role in PON1 inactivation [23,24,39,40]. Another possible mechanism of diminished PON1 activity in HD patients involved Hcy and/or its metabolite. Plasma Hcy, protein-*N*-homocysteinylation and protein-*S*-homocysteinylation were significantly higher in HD patients compared with controls [41].

In the current study, the decrease in PON1 lactonase was most obvious in HD patients with thrombosed AV fistulas compared with patients with non-thrombosed AV fistulas. Our results indicate that serum PON1 lactonase activity may identify HD patients at risk for future native AV fistula thrombosis. Previously, it was shown that PON1 paraoxonase and lactonase activities were decreased while the cardiovascular disease risk factors, including Hcy, cystatin C, asymmetric dimethyl arginine and CRP were increased, in HD patients [25]. These results indicate that PON1 lactonase might be one of the predictors of cardiovascular disease risk in HD patients. Moreover, PON1 paraoxonase and lactonase activities were found to be significantly decreased in CAD patients compared

with controls. In the CAD patients, a negative correlation was observed between paraoxonase and lactonase activities and the extent of atherosclerosis. HDL concentration was decreased and both PON1 activities were positively correlated with HDL levels in the CAD patients [27]. Given the hypothesis that PON1 is a potential contributing factor for the prevention of atherosclerosis, and possibly the incidence of native AV fistula thrombosis in HD patients, there is an immense need to find strategies for increasing PON1 activity and/or expression. Hypolipemic drugs (statins and fibrates) and dietary factors (antioxidants and polyphenols) have been shown to increase PON1 activity [42].

Interestingly, we found that serum FGF-23 level was significantly increased in HD patients with more than two episodes of AV fistula thrombosis compared with patients with one episode. Moreover, we found that serum PON1 lactonase activity was significantly decreased in HD patients with either two episodes or more than two episodes of AV fistula thrombosis compared with HD patients with only one episode. Moreover, a significant inverse correlation was found between FGF-23 level and PON1 lactonase activity in thrombosed HD patients, and a similar but less significant trend was detected in non-thrombosed HD patients.

Despite numerous publications on biomarkers, very few tests have been added to the routine repertoire of clinical biochemistry laboratories. Several points need to be clarified before circulating FGF-23 level and PON1 lactonase activity may be considered as new biomarkers in the routine care of HD patients. Firstly, it is crucial to compare the reliability of the studied markers with existing best practice in the population for which it is intended. Secondly, we need to study the effect of medications that might be used to treat the patients in whom the biomarkers will be measured. Thirdly, the diagnostic industry needs to offer cost-effective reliable tools.

Although the results of this study are promising, caution should be used before the data can be used in other clinical settings and with other populations. The study included only Egyptian patients; thus, the findings may not apply to other patients. In addition the patients studied were from a single clinical centre. Finally the sample size was relatively small.

In conclusion, detection of high level of serum FGF-23 and low activity of serum PON1 lactonase may help to predict the occurrence of native AV fistula thrombosis in HD patients. More research studies, in different clinical settings on a large number of HD patients that have thrombosed and

non-thrombosed AV fistulas, are needed to verify our observations and allow general recommendations to be made.

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