In end-stage renal disease, the effects of hemodialysis on chronic fatigue syndrome- and fibromyalgia-like symptoms are mediated via inflammatory biomarkers, copper and Wnt/catenin pathway proteins.

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https//:scholar.google.co.th/citations?user=1wzMZ7UAAAAJ&hl=th&oi=ao

Abstract

End-stage renal disease (ESRD) is associated with fatigue and physio-somatic symptoms. The aims of this study are to delineate the associations between severity of fatigue and physio-somatic symptoms and glomerular filtration rate, inflammatory biomarkers, and Wnt/catenin-pathway proteins. The Wnt-pathway related proteins β-catenin, Dickkopf-related protein 1 (DKK1), R-spondin-1, and sclerostin were measured by ELISA technique in 60 ESRD patients and 30 controls. The Fibromyalgia and Chronic Fatigue Syndrome (FF) Rating Scale was used to assess severity of FF symptoms. ESRD is characterized by a significant increase in the total FF score, muscle tension, fatigue, sadness, sleep disorders, GI symptoms, and a flu-like malaise. The total FF score was significantly correlated with serum levels of urea, creatinine, phosphate, and copper (positively), and β-catenin, eGFR, hemoglobin, albumin, and zinc (inversely). The total FF score was associated with the number of total dialysis and weekly dialysis sessions, and these dialysis characteristics were more important in predicting FF scores than eGFR measurements. Partial Least Squares analysis showed that the FF score comprised two factors which are differently associated with biomarkers: a) 43.0% of the variance in fatigue, GI symptoms, muscle tension, sadness, and insomnia is explained by hemoglobin, albumin, zinc, β-catenin, and R-spondin-1; and b) 22.3% of the variance in irritability, concentration and memory impairments by increased copper and cations/chloride ratio, and male sex. ESRD patients show high levels of fatigue and physio-somatic symptoms which are associated with hemodialysis and mediated by dialysis-induced changes in inflammatory pathways, the Wnt/catenin pathway, and copper.


Keywords: Myalgic Encephalomyelitis/chronic fatigue syndrome, Wnt pathway, inflammation, neuro-immune, oxidative stress, biomarkers.

## Introduction

Acute kidney injury (AKI) occurs in approximately 10–15% of patients admitted to hospital [1] and its incidence in the intensive care unit has been reported to be 12.6-12.9% [2]. AKI is characterized by a rapid deterioration in kidney functions and may lead to end-stage renal disease (ESRD) [3]. When AKI patients are left untreated for a continued period, irreversible tubular necrosis may result in progress to ESRD [4]. ESRD is a severe irreversible decline in kidney function, which may lead to death in the absence of dialysis or kidney transplantation [5]. A recent study found that 70.7% of ESRD patients undergo dialysis and that 29.3% needed a kidney transplant [6].

ESRD patients experience a multitude of mental and physio-somatic symptoms including depression, anxiety, fatigue, fibromyalgia-like symptoms, muscular pain, insomnia, headache, and cognitive impairments [7-13]. The prevalence of fatigue in renal disease ranges between 42-89% depending on the rating scales used [14]. In ESRD patients, fatigue may impact the work abilities, social interactions as well as the quality of life [15, 16]. In ESRD, fatigue and physio-somatic symptoms are not regularly assessed and, therefore, these symptoms are frequently underestimated [17]. Given the impact of fatigue and physio-somatic symptoms on the overall health state and health-related quality of life of ESRD patients, it is important to delineate the pathophysiology of those symptoms and delineate new drug targets that could help to treat those symptoms that accompany ESRD and hemodialysis. Nevertheless, the biomarkers of fatigue and physio-somatic symptoms in ESRD and hemodialysis are not fully explored.

Myalgic Encephalomyelitis / chronic fatigue syndrome (ME/CFS) is a disorder characterized by symptoms that also occur in ESRD, including depression, fatigue, fibromyalgia-like symptoms, muscular pain, insomnia, headache, and cognitive impairments [18, 19]. There is

evidence that ESRD and ME/CFS are both characterized by a multitude of intertwined pathways including activation of immune-inflammatory and nitro-oxidative stress pathways.

Chronic kidney disease (CKD) and ESRD are accompanied by increased levels of various pro-inflammatory cytokines and acute phase proteins including C-reactive protein (CRP) [20-22], biomarkers of oxidative stress [23, 24] including lowered zinc, and disorders in trace elements such as increased copper [25]. The progression of CKD is associated with activated immune-inflammatory and nitro-oxidative stress pathways which additionally may cause heart failure, atherosclerosis, malnutrition, anemia and overall mortality [20]. Furthermore, hemodialysis itself is accompanied by intertwined increases in inflammation and oxidative stress which are associated with cardiovascular events [26, 27]. Most if not all patients with CKD under hemolysis suffer from the anemia of chronic disease with accompanying lowered hemoglobin levels, which are at least in part due to persistent inflammation [28, 29]. Another biomarker of CKD and hemodialysis is hypoalbuminemia which may be explained by chronic inflammation (albumin is a negative acute phase protein), malnutrition, and lowered residual renal activity [30, 31]. In hemodialysis patients, zinc concentrations may be negatively correlated with serum copper levels, which are often increased in CKD [25, 32]. Finally, malfunctions of electrolyte channels and transporters in the injured kidneys may cause abnormalities in sodium, potassium, chloride, and phosphate [33-35].

ME/CFS is an immune-inflammatory and oxidative stress disorder which is characterized by increased levels of pro-inflammatory cytokines, an acute phase response with lowered levels of negative acute phase reactants such as albumin and zinc, and increased oxidative stress [18, 19, 36]. In addition, the severity of ME/CFS, as assessed with the Fibromyalgia and Chronic Fatigue Syndrome (FF) Rating Scale [37], is associated with signs of activated immune-inflammatory and oxidative pathways [38]. Previously, it was discussed that these pathways may cause symptoms of

mental and physical fatigue and the physio-somatic symptoms including muscle fatigue and pain, insomnia, hyperesthesia, gastro-intestinal (GI) symptoms, cognitive impairments and affective symptoms as well [18, 19, 36, 39, 40].

The Wnt/catenin pathway intersects with immune-inflammatory and oxidative pathways and plays a key role in early nephrogenesis and the onset of various kidney disorders and chronic kidney disease [41-43]. Activation of the Wnt/β-catenin pathway plays an important role in the repair and regeneration of renal tubules after AKI [44]. The major players in the Wnt pathway are the antagonists Dickkopf-related protein 1 (DKK1) and sclerostin (SOST) [45], the agonist R-spondin 1 (RSPO1), which amplifies Wnt signaling [46] and attenuates the inhibition imposed by DKK1 on the Wnt-pathway [47], and the effector molecule β-catenin, which functions as a component of the cadherin complex, thereby controlling cell-cell adhesion [48]. Inflammation is accompanied by an elevated expression of DKK1, a pro-inflammatory glycoprotein secreted by endothelial cells and platelets [49]. As an antagonist of the canonical Wnt signaling pathway, DKK1 may interfere with tissue repair and regeneration and cause neurotoxicity, decreased neurogenesis, synaptic loss, and a rapid disassembly of synapses in mature neurons [49-53]. Increased DKK1 circulating levels are associated with cognitive impairments in elderly individuals [54] and lowering DKK1 in animal models improves affective behaviors and cognition [53]. Moreover, the Wnt pathway regulates the blood-brain barrier (BBB), with DKK1 inducing BBB breakdown, and β-catenin promoting the permeability of the BBB endothelial cells [55-58]. Such effects may explain that, in schizophrenia, increased levels of serum DKK1 are positively associated with signs of inflammation, and chronic fatigue and fibromyalgia-like symptoms [59, 60]. Nevertheless, there are no data on a possible association between Wnt pathway proteins and the fatigue and physio-somatic symptoms in AKI-associated ESRD.

Hence, this study was conducted to examine whether hemodialysis or CKD/ESRD may affect fatigue and physio-somatic symptoms in ESRD and whether these effects are mediated by biomarkers of lowered kidney functions (eGFR, urea and creatinine), inflammation (albumin, zinc, hemoglobin), the Wnt/catenin pathway (measurements of the 4 main proteins), phosphate, copper, and/or electrolyte changes.

**Participants and Methods**

*Participants*

The present study recruited sixty ESRD patients (30 male and 30 female) aged 15-55 years who had a previous AKI and developed renal failure. All patients were on continuous dialysis and were patients at the Al-Sader Medical City and the Dialysis Unit at Al-Hakeem General Hospital in Najaf Governorate-Iraq. They were examined during the period October-December 2020. The evaluation of ESRD patients was based on a full medical history and the diagnosis of ESRD was made according to the 10th revision of the International Statistical Classification of Diseases and Related Health Problems (2021 ICD-10-CM Diagnosis Code N18.6). ESRD represents the 5th stage of renal failure with eGFR < 15 mL/min/1.73 $m^2$ [61]. All patients were on continuous treatment with folic acid or iron and folate formula (Fefol®) in addition to calcium carbonate, Epoetin alfa (Eprex®) and heparin. Exclusion criteria were the absence of any other systemic disease, including diabetes and liver or heart diseases. Serum C-reactive protein (CRP) concentrations were < 6 mg/L in all participants as assayed using an agglutination test, thereby excluding overt inflammation. The control group comprised 30 healthy control subjects without apparent physical illnesses, namely 15 males and 15 females. They were sex and age matched with the patients. Written informed consent was obtained from all controls and the patients or their first-

degree relatives. The protocol was approved by the Iraqi institutional review board (IRB) of the University of Kufa (543/2020), Kufa, Iraq.

*Fibromyalgia and Chronic Fatigue Syndrome Rating*

A senior psychiatrist assessed the Fibromyalgia and Chronic Fatigue Syndrome Rating (FF) scale to measure the severity of CFS- and fibromyalgia-like symptoms [62]. "The FF scale measures 12 symptoms, namely FF1: muscle pain, FF2: muscular tension, FF3: fatigue, FF4: concentration difficulties, FF5: failing memory, FF6: irritability, FF7: sadness, FF8: sleep disturbances, FF9: autonomic disturbances, FF10: irritable bowel, FF11: headache, and FF12: a flu-like malaise. The total sum of all 12 items was used as an index of overall severity of fatigue and physio-somatic symptoms" [63]. Tobacco Use Disorder (TUD) was diagnosed using DSM-IV-TR criteria. The Body mass index (BMI) was assessed on the same day as the clinical interview as body weight in kg/length in $m^2$.

*Measurements*

Fasting (overnight fast) venous blood was sampled between 8.00-9.00 a.m and collected into plain tubes. Serum was sampled before the hemodialysis session to assay all parameters. After separation, the sera were distributed into three new Eppendorf® tubes for further analysis. Serum sclerostin (SOST), R-Spondin 1 (RSPO), β-catenin (CAT), and DKK1 concentrations were measured using ELISA kits supplied by Melsin Medical Co., Ltd., Jilin, China. The interassay CV% of all the Wnt-pathway proteins were <15%. All measured concentrations of sclerostin (sensitivity = 0.1 ng/ml), R-spondin1 (sensitivity = 1 pg/mL), β-catenin (sensitivity = 1 pg/mL), and DKK1 (sensitivity = 1 ng/mL) were greater than the sensitivity of the assays.

The electrolyte (Na$^+$, K$^+$, and Cl$^-$) concentrations were measured in serum by using the HumaLyte Plus[5] ion selective electrolyte analyzer (HUMAN Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany). Consequently, we computed a z unit weighted composite score based on the strong cations, Na, and K, and the strong anion Cl after z transformation. As such this index (Cations/Chloride) reflects the strong ion difference. Hematological biomarkers were measured using a five-part differential Mindray BC-5000 hematology analyzer (Mindray Medical Electronics Co., Shenzhen, China). Serum copper and zinc were measured spectrophotometrically using kits supplied by Spectrum Diagnostics Co. (Cairo, Egypt). Glucose, albumin, urea, and creatinine were measured spectrophotometrically by ready for use kits supplied by Biolabo® (Maizy, France). The estimated GFR (eGFR) was calculated by using the Modification of Diet in Renal Disease (MDRD) study equation [64] using the following formula:

$$eGFR = 175 \times (S.Cr)^{-1.154} \times (Age)^{-0.203} \times 0.742 \text{ [if female]} \times 1.212 \text{ [if Black]}$$

*Statistical Analysis*

Analysis of variance (ANOVA) was employed to investigate the between-group differences in scale variables. Statistical associations between categorical variables were checked using analysis of contingency tables ($\chi^2$-test). Pearson's correlation coefficients (r) or Spearman's correlation coefficients ($\rho$, rho) were calculated to assess correlations between biomarkers and the FF scale score and dialysis characteristics. We Ln transformed biomarkers to normalize the distribution as assessed using the Kolmogorov-Smirnov test. We employed multivariate generalized linear model (GLM) analysis to assess the relationship between the biomarkers and the diagnosis (ESRD versus controls) while controlling for background variables including age,

BMI, TUD, and sex. Tests for between-subject effects or univariate GLM were performed to assess the associations between diagnostic classes and biomarkers and the effect size was estimated with partial eta-squared. Binary logistic regression analysis was used to delineate the most important variables which predict ESRD versus controls (used as the reference group). We employed multiple regression analysis to check the most significant biomarkers explaining the total FF score or its subdomains while allowing for the effects of possible intervening variables such as sex, age, TUD, and BMI. All results were checked for $R^2$ change and multicollinearity using VIF and tolerance values. Statistical tests were 2-tailed, and a p-value of 0.05 was used for statistical significance. All statistical analyses were performed using IBM SPSS windows version 25, 2017. Power analysis showed that using an effect size of 0.3, alpha=0.05, power=0.8 and two groups, the total sample size should be at least 90.

Unweighted least Squares Factor analysis using 500 bootstrapped samples was performed using FACTOR 10.10.01 x64bits [65]. Optimal implementation of parallel analysis was used to determine the number of factors, and varimax rotation to interpret the factors. The Adjusted Goodness of Fit Index (AGFI), Bentler's simplicity index, and the Root Mean Square of Residuals (RMSR) were used to evaluate the model quality [66]. Construct reliability was assessed using the Generalized H index with H values >0.80 indicating well defined latent variables. The quality and effectiveness of the factor score estimates were assessed using the Factor Determinacy Index (FSI), the marginal reliability ORION (Overall Reliability of fully-Informative prior Oblique N-EAP scores), the Sensitivity Ratio (SR), and Expected Percentage of True Differences (EDTD). FDI > 0.90, marginal reliabilities > 0.80, SR > 2, and EPTDs > 90% indicate that factor scores may be used for individual assessment.

We employed Partial least squares (LSD) analysis [67] to examine the associations between ESRD/hemodialysis and the biomarkers which were entered as input variables, and FF symptoms which were entered as output variables. R-spondin 1, β-Catenin, zinc, copper, Cation/ Chloride ratio, and FF item 12 were entered as single indicators (or as a latent-vectors extracted from FF symptom dimensions, inflammatory markers, and dialysis features. PLS path analysis was performed only when: a) all indicators of all latent vectors loaded highly (>0.500) at p<0.001 on that vector; b) the latent vectors had adequate reliability as indicated by composite reliability > 0.7 and average variance extracted (AVE) > 0.500; c) the quality of the model fit is adequate as indicated by Standardized Root Mean Squared Error (SRMR) < 0.080. Consequently, we performed complete PLS analysis on 5.000 bootstrap samples and computed path coefficients with p-values, specific indirect effects and total effects [68].

Results

*Demographic and clinical data*

The sociodemographic and clinical data of ESRD patients and healthy controls are presented in **Table 1.** There were no significant differences in age, sex ratio, marital status, TUD, family history of CKD, and rural/urban ratio between the study groups. ESRD patients had a significantly lower BMI and employment ratio than healthy controls. There is a significant increase in the scores of muscle tension, fatigue, sadness, sleep disorders, GI symptoms, a flu-like malaise, total FF score in ESRD as compared with controls. The scores of muscle pain, concentration disorders, memory disturbances, irritability, and headache did not show significant differences between both study groups.

*Differences in biomarkers between ESRD and healthy controls*

　　**Table 2** shows results typical of the ESRD condition, namely low eGFR values, significant increaseₛ in serum urea and creatinine, and low hemoglobin, albumin, and zinc, and higher inorganic phosphate and copper. We found a significant decrease in the ratio of Cations / Chloride in ESRD. The same Table shows the measurements of the Wnt-pathway proteins in both ESRD and controls. We found a significant increase in DKK1 and sclerostin in ESRD as compared with controls. β-catenin was significantly lowered in ESRD as compared with controls.

*Results of exploratory factor analysis*

　　**Table 3** shows the results of an unweighted least squares factor analysis performed on all FF items (except autonomic symptoms, which did not show any variance). Parallel analysis showed that the advised number of dimensions is two. The first two factors explained 52.4% of the variance. The adjusted goodness of fit index was 0.951, Bentler's Simplicity Index 0.992 (97th percentile), and root mean square of residual (RMSR) 0.080 (Kelley's criterion expected mean value of RMRS for an acceptable model was 0.1060), indicating an adequate fit. Table 3 shows that the construct replicability indices, and that the quality and effectiveness of factor score estimates were adequate. Interpretation of the varimax-rotated factors showed that factor 1 loaded highly on muscle pain, muscle tension, fatigue, sadness, sleep disorders, GI symptoms, and headache, and that factor 2 loaded highly on concentration and memory complaints and irritability. Consequently, we have used both factor scores as well as the total sum of their items as dependent variables in various analyses. Table 1 shows that the sum of the 7 factor 1 items (sum FF Factor1) was significantly higher in ESRD patients than in controls. The sum of the three F2 items (sum FF Factor2) did not differ between both groups.

*Intercorrelation matrix of the FF scores, dialysis characteristics and biomarkers*

The intercorrelation matrix of FF scores, clinical characteristics and biomarkers is presented in **Table 4**. The total FF and FF Factor1 scores were significantly and positively correlated with total and weekly number of dialysis sessions, urea, creatinine, phosphate, and copper, and inversely with eGFR, hemoglobin, albumin, zinc, and β-catenin. The FF Factor1 score was correlated with duration if illness. The sum FF Factor2 score was significantly and positively associated with number of dialysis / week and the Cations / Chloride ratio. There were no significant correlations between the FF scores and DKK1, R-spondin 1, and sclerostin.

*Multiple regression analysis with total FF scores*

**Table 5** shows the results of different stepwise multiple regression analyses with the total FF score as dependent variable and biomarkers and clinical characteristics as independent variables while allowing for the effects of age, sex, TUD, and BMI. These associations were examined in the total study group and in patients separately. Regression #1 shows that, in the total study group, 38.3% of the variance in the total FF score was explained by the regression on eGFR (inversely associated) and Cations / Chloride ratio (positively associated). Regression #2 shows that 42.7% of the variance in the total FF score could be explained by the regression on urea and Cations /Chloride ratio (positively associated). **Figure 1** shows the partial regression plot of the total FF score on serum urea. Regression #3 shows that 39.5% of the variance in the total FF score was explained by R-spondin 1 and age (positively associated), and serum hemoglobin and β-Catenin (both inversely associated). Regression #4 shows that, in ESRD

patients, 33.8% of the variance in the total FF score was explained by the weekly number of

dialysis sessions, R-spondin 1 (positively associated) and β-Catenin (inversely associated).

*Multiple regression analysis with the FF Factor scores*

The results of multiple regression analyses with FF Factor1 and2 scores as the dependent

variables and Wnt-pathway proteins and ESRD-related biomarkers as explanatory variables are

presented in **Table 6.** Regression #1 shows that a considerable part of the variance in FF Factor1

score (41.5%) was explained by the regression on urea (positively associated) and regression #2

shows that 39.2% of the variance in the FF Factor1 score can be explained by the regression on

eGFR (inversely associated). Regression #3 shows that a significant part of the variance (41.7%)

in the FF Factor1 score may be explained by the regression on hemoglobin and β-catenin (inversely

associated) and age and R-spondin (positively associated). **Figure 2** shows the partial regression

of the FF Factor1 score on hemoglobin concentrations. **Figure 3** shows the partial regression of

the FF Factor1 score on β-Catenin concentrations. Regression #4 shows that 29.5% of the variance

in FF Factor2 could be explained by the regression on age, sex, Cations / Chloride ratio (positively

associated) and BMI (inversely associated). **Figure 4** shows the partial regression of FF Factor 2

score on the zCations/Cl ratio.

*Results of PLS analysis*

**Figure 5** displays the results of PLS pathway analysis with both FF symptom subdomains

(FF Factor1 and FF Factor2 and FF item 12) as output variables, and dialysis features (duration of

dialysis, total and weekly number of dialyses) and biomarkers as input variables. The latent vector

based on eGFR, urea and creatinine levels were no longer significant as explanatory variable after

entering the dialysis latent vector. This PLS model showed an adequate fit with SRMR=0.059; all indicators of the outer model showed loadings > 0.500 (at p<0.001); and all 4 latent vectors included in the model showed adequate reliability with composite reliability > 0.830 and AVE > 0.574. We found that 43.0% of the variance in the FF Factor1 latent vector was explained by the regression on the inflammation latent vector, β-catenin, R-spondin 1, and zinc; that 10.2% of the variance in FF item 12 was explained by the inflammation latent vector; and 22.3% of the variance in the FF Factor2 by the Cations / Chloride ratio, copper and male sex. There were specific indirect effects of hemodialysis on FF Factor1 (t=4.38, p<0.001), which were mediated by the inflammation latent vector (t=4.38, p<0.001) and lowered catenin levels (t=2.02, p=0.044). There were specific indirect effects of hemodialysis on FF Factor2, which was mediated by increased copper (t=2.38, p=0.017), and on FF item 12, which was mediated by the inflammation laten vector (t=3.29, p=0.001). There were significant total effects of hemodialysis on FF Factor1 (t=7.00, p<0.001), FF Factor 2 (t=2.38, p=0.017) and FF item 12 (t=3.29, p=0.001).

Discussion

*Fatigue and physio-somatic symptoms in ESRD/hemodialysis*

The first major finding of this study is that ESDR patients have higher scores on the total FF and on the FF items muscle tension, fatigue, sadness, sleep disorders, GI symptoms, and a flu-like malaise as compared with healthy controls. These findings indicate that ESRD is accompanied by subchronic increases in fatigue, and physio-somatic and depression-like symptoms.

Patients with ESRD and those undergoing long-term dialysis emphasize fatigue and exhaustion as the most significant symptoms [16, 69-72]. Fatigue is probably the most prevalent symptom in renal disease and hemodialysis patients [14]. Moreover, in those undergoing long-

term hemodialysis, more than 50% of the patients suffer from fatigue symptoms [73, 74], lack of physical energy, physical and mental fatigue, cognitive impairments and reduced day to day activities [11, 69, 75]. In dialysis patients, depression is another common manifestation that is associated with fatigue [11, 76, 77]. In dialysis patients, insomnia is associated with increased fatigue [71] and exhaustion [78]. Insomnia and muscle cramps are among the top-3 most frequently reported physio-somatic symptoms in ESRD [8]. Chronic musculoskeletal pain, muscle weakness and cramps, and insomnia are frequently observed in patients with CKD [79].

Interestingly, our patients with ESRD did not suffer from concentration and memory impairments. Likewise, our factor analysis detected two interpretable factors, a first factor with symptoms that are associated with ESRD (fatigue and physio-somatic) and a second with concentration and memory impairments and irritability. This contrasts with other disorders including ME/CFS, schizophrenia, and major depression where all these symptoms are significantly increased [59, 63, 80-82]. Previously, it was observed that hemodialysis patients may experience cognitive fatigue with a decline in cognitive capacity as indicated by memory impairments including in recall [11, 69, 75]. It is also interesting to note that the factor score extracted from cognitive symptoms and irritability is higher in men than in women. In a previous study, female patients on dialysis reported higher fatigue levels than males [83, 84], although our study did not find a significant effect of sex on fatigue ratings.

The current study also established that severity of the FF symptoms was significantly associated with the total number of hemodialysis sessions and even more with the weekly number of dialysis sessions. Previously, it was reported that patients with prolonged periods of hemodialysis treatment feel more fatigued [85]. On the other hand, frequent hemodialysis sessions may be associated with reduced fatigue and an improved patients' quality of life [86, 87]. Patients

who recently started dialysis treatment sometimes report more fatigue as compared with patients who were treated for longer periods [88]. In hemodialysis patients, the fatigue often worsens on the day of dialysis treatment [11, 75]. It should be noted that patients with hemodialysis also experience an aggravation of the fatigue during and after the dialysis therapy, a phenomenon labeled "post-dialysis fatigue" [89].

*Biomarkers of FF symptoms in ESRD and hemodialysis*

Fatigue and physio-somatic symptoms in ESRD are often ascribed to complications of ESRD, side effects of the medications used to treat chronic kidney disease, and to the comorbidities of chronic kidney disease [11, 75]. These symptoms are also ascribed to negative emotions and perceived threats of long-term dialysis treatment and the perception of depression [11]. Other factors that may affect fatigue in dialysis patients are age, sex, and ethnicity [71, 90, 91]. Nevertheless, in the current study we found that a large part of the variance in ESRD-associated FF symptoms (namely FF Factor1 symptoms) was explained by ESRD-related biomarkers (see Introduction) and by alterations in Wnt/catenin pathway proteins. The current study found that the fatigue and physio-somatic symptoms of ESRD were strongly associated with the typical ESRD status biomarkers namely increased urea, creatinine, inorganic phosphate, and copper, and inversely with eGFR, hemoglobin, albumin and zinc. In previous work, low eGFR was associated with fatigue in dialysis patients [92]. Classical uremic symptoms are fatigue, extreme tiredness, anergy, muscle weakness and cramps, insomnia, and headache especially when the urea concentrations are > 300 mg/dL [10-13]. Feeling weak and fatigue are also frequently attributed to increased creatinine concentrations [93]. Increased inorganic phosphate is a major cause of muscle fatigue [94, 95] and severe musculoskeletal pain, especially in stage 5 CKD [96]. Fatigue

and muscular pain in severe CKD patients may be explained by a number of factors including persistent inflammation (as indicated by lowered albumin, zinc and hemoglobin), malnutrition, and the anemia of chronic disease [14, 79].

Chronic inflammation is common in patients with ESRD [97] and is associated with fatigue and lowered energy levels in patients on dialysis [71, 98]. Higher levels of pro-inflammatory cytokines can lead to fatigue in both dialysis and kidney transplants recipients [99]. ME/CFS is associated with lowered levels of serum zinc, which is related to inflammatory and immune biomarkers [100]. There are many mechanistic explanations why inflammation and its consequences may cause fatigue and physio-somatic symptoms as reviewed in [18, 19, 39, 40].

Although inflammation may be caused by the ESRD, dialysis therapy may aggravate inflammation, because exposure of the blood of the patient to the dialysis membrane and tubing may induce an inflammatory response [101]. In the present study, both lowered eGFR (and hyperuremia/hypercreatininemia) and hemodialysis were associated with changes in the ESRD-associated biomarkers of inflammation, phosphate, and copper. Nevertheless, PLS pathway analysis indicated that hemodialysis was more important than lowered eGFR (and hyperuremia/hypercreatininemia) in predicting the FF Factor1 and item FF12 (a flu like malaise) scores. Also, the timely connections between number of weekly dialysis sessions and FF symptoms indicate that dialysis is an important determinant of FF symptoms in ESRD patients. Our PLS model detected that the effects of dialysis on the FF scores were mediated by inflammation, copper, and Wnt proteins (see below). Nevertheless, these statistical findings do not rule out that hyperuremia and hypercreatininemia may contribute to FF symptoms.

It is interesting to note that concentration and memory disorders and irritability were strongly associated with the Cations / Chloride ratio, an index of a strong ion difference. It should

be stressed that the latter was affected by weekly number of dialysis sessions, suggesting that dialysis may induce disorders in this ratio leading to Factor2 symptoms. Previously, associations were reported between the anion gap and amnestic mild cognitive impairment [102] and delirium [103]. Interestingly, increased serum copper levels mediated the effects of dialysis on cognitive complaints and irritability.

*The Wnt/catenin pathway and FF symptoms in ESRD*

Another major finding of this study is that fatigue and physio-somatic symptoms in ESRD are associated with Wnt/catenin pathway proteins. However, contrary to the primary hypothesis no associations were found with the antagonists DKK1 and sclerostin. On the contrary, we found that the total FF score was inversely associated with β-catenin and positively with R-spondin 1, which together with number of weekly dialysis sessions explained a large part of the variance in the total FF score. Interestingly, when we performed the statistical analysis in patients with ESRD, both β-catenin and R-spondin 1 and weekly dialysis sessions were more important predictors of the total FF score than eGFR, urea, creatinine, and the inflammatory biomarkers. This indicates that these Wnt pathway proteins have an effect on fatigue and physio-somatic symptoms above and beyond the effects of urea, creatinine and inflammation.

R-spondin-1 is an endogenous ligand potentiating Wnt signaling [104, 105] and stimulating the renewal of Wnt-dependent stem cells to maintain tissue homeostasis in many different tissues [106-108]. Moreover, Wnt agonists may reduce creatinine and BUN levels, inflammatory responses and oxidative stress [109]. On the other hand, activation of the Wnt/catenin pathway may contribute to kidney injuries through excessive extracellular matrix deposition and upregulation of fibrosis-associated genes thereby worsening CKD [110]. Lowered

levels of β-catenin could play a role in the breakdown of the BBB (see introduction), thereby aggravating the effects of inflammation on brain tissues involved in fatigue and physio-somatic symptoms [39, 40]. Moreover, lowered β-catenin may modulate cellular functions resulting in lowered resilience to stress through miRNA upregulation [111] and, in the brain, β-catenin may mediate synaptic plasticity, neuroplasticity and memory consolidation [111].

Limitations

The results of this paper should be discussed with regard to its strengths and limitations. Firstly, we employed a cross-sectional design which does not allow to draw firm causal associations. Secondly, it would have been even more interesting if we had measured cytokine profiles, positive acute phase proteins and bicarbonate. The construction of a PLS path model is one of the strengths of this study because it displays how inflammatory and Wnt/catenin biomarkers and copper mediate the effects of hemodialysis on fatigue and physiosomatic symptoms.

Conclusions

Patients with ERSD with hyperuremia and hypercreatininemia show significant increases in fatigue and selected physio-somatic symptoms including muscle aches, headache, sadness, GI symptoms, insomnia and malaise, but not cognitive impairments and irritability. PLS pathway analysis shows that the effects of dialysis (total and weekly number) on muscle aches, headache, sadness, GI symptoms, insomnia and malaise are mediated by signs of inflammation (lowered albumin and hemoglobin) and lowered zinc, and changes in Wnt/catenin pathway proteins, namely increased R-spondin 1 and lowered β-catenin; and the effects of dialysis on malaise are mediated

by inflammation. The cognitive impairments and irritability are mediated by ESRD-related increases in copper and increases in strong ion differences. All in all, fatigue and physio-somatic symptoms in ESRD appear to be mediated by multiple pathways.

## Acknowledgment

## Funding

## Conflict of interest

The authors have no conflicts of interest with any industrial or other association with reference to the submitted article.

## Author's contributions

All the contributing authors have participated in the preparation of the manuscript.

## Consent to participate.

All controls and patients as well as their guardians (parents or other close family members) gave written informed consent prior to participation in this study.

## Data availability statement

The database generated during this study will be made available from the corresponding author on reasonable request once the data set has been fully exploited by the authors.

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Table 1. Sociodemographic and clinical data and measurements of the Fibromyalgia and Chronic Fatigue Syndrome (FF) Rating Scale in patients with end-stage renal disease (ESRD) and healthy controls (HC).

| Parameter | HC (n=30) | ESRD (n=60) | F/FEPT/$\chi^2$ | df | p |
|---|---|---|---|---|---|
| Age            (years) | 32.0±9.2 | 33.1±11.9 | 0.20 | 1/88 | 0.653 |
| Sex     (Female/Male) | 15/15 | 30/30 | 0.00 | 1 | 1 |
| Single/married | 8/22 | 26/34 | 2.36 | 1 | 0.124 |
| TUD            (No/Yes) | 28/2 | 57/3 | - | - | 1.00 |
| Urban/Rural | 26/4 | 41/19 | 3.53 | 1 | 0.060 |
| Employment    (No/Yes) | 3/27 | 57/3 | - | - | <0.001 |
| Family history of CKD (No/Yes) | 30/0 | 58/2 | - | - | |
| BMI            (kg/m$^2$) | 25.0±2.5 | 22.8±3.7 | 8.74 | 1/88 | 0.004 |
| Duration of illness (months) | - | 28.30±33.25 | - | - | - |
| Dialysis session /week | - | 2.05±0.52 | - | - | - |
| Total dialysis sessions | - | 287±381.05 | - | - | - |
| FF1: Muscle pain | 1.13±0.63 | 1.23±0.89 | 0.30 | 1/88 | 0.584 |
| FF2: Muscle tension | 0.03±0.18 | 0.35±0.66 | 6.63 | 1/88 | 0.012 |
| FF3 Fatigue | 0.43±0.57 | 1.10±0.78 | 17.47 | 1/88 | <0.001 |
| FF4: Concentration Disorders | 0.03±0.18 | 0.17±0.56 | 1.62 | 1/88 | 0.206 |
| FF5: Memory disturbances | 0.03±0.18 | 0.13±0.47 | 1.27 | 1/88 | 0.264 |
| FF6: Irritability | 0.23±0.43 | 0.40±1.01 | 0.74 | 1/88 | 0.391 |
| FF7: Sadness | 0.07±0.25 | 1.32±0.62 | 110.66 | 1/88 | <0.001 |
| FF8: Sleep disorders | 0.33±0.48 | 1.72±0.99 | 51.94 | 1/88 | <0.001 |
| FF10: Gastro-intestinal symptoms | 0.43±0.50 | 1.52±1.24 | 21.00 | 1/88 | <0.001 |
| FF11: Headache | 0.47±0.63 | 0.57±0.83 | 0.34 | 1/88 | 0.563 |
| FF12: Flu-like malaise | 0.30±0.47 | 0.83±0.79 | 11.74 | 1/88 | 0.001 |
| FF-Total* | 3.50±1.72 | 9.33±4.93 | 59.33 | 1/88 | <0.001 |
| FF Factor1 score | -0.784±0.366 | 0.392±0.855 | 51.74 | 1/88 | <0.001 |
| FF Factor2 score | -0.107±0.398 | 0.054±1.179 | 1.52 | 1/88 | 0.220 |

Results are shown as mean ±SD. All results of analysis of variance (F), Fisher's Exact probability test (FEPT) or analysis of contingency analysis ($\chi^2$-test). BMI: body mass index, TUD: tobacco use disorder. *Processed in Ln transformation.

Table 2. Biomarkers of end-stage renal disease (ESRD) as compared with healthy controls (HC).

| Parameter | HC (n=30) | ESRD (n=60) | F | p |
|---|---|---|---|---|
| estimated glomerular filtration rate mL/min* | 129.9±63.3 | 7.8±3.5 | 967.40 | <0.001 |
| Urea          mg/dl | 29.07±6.45 | 170.40±55.78 | 190.28 | <0.001 |
| Creatinine     mg/dl | 0.72±0.24 | 8.04±2.44 | 267.34 | <0.001 |
| Hemoglobin    g/dl | 13.39±1.42 | 8.11±1.71 | 212.01 | <0.001 |
| Albumin        g/l | 44.30±5.85 | 37.87±5.92 | 23.83 | <0.001 |
| Inorganic Phosphate mM | 1.30±0.15 | 1.980±0.40 | 79.63 | <0.001 |
| Glucose        mg/dl | 94.0±10.8 | 95.8±19.7 | 0.22 | 0.643 |
| Cations / Chloride (z scores) | 0.299±0.590 | -0.269±1.387 | 4.58 | 0.035 |
| Zinc            mg/l | 0.73±0.26 | 0.55±0.29 | 9.12 | 0.003 |
| Copper          mg/l | 0.81±0.14 | 1.51±0.75 | 25.81 | <0.001 |
| Dickkopf protein 1  ng/ml | 18.69±10.95 | 26.33±16.66 | 5.19 | 0.025 |
| R-Spondin 1  pg/ml | 240.5±111.0 | 216.1±72.0 | 1.58 | 0.213 |
| Sclerostin      ng/ml | 3.60±1.67 | 4.43±1.73 | 4.75 | 0.032 |
| β-Catenin      pg/ml | 114.8±66.4 | 77.1±39.4 | 11.39 | 0.001 |

Results are shown as mean ±SD. All results of analyses of variance (df=1/88).

Table 3. Results of unweighted least squares factor analysis performed on the items of the Fibromyalgia and Chronic Fatigue Syndrome (FF) Rating Scale.

| FF items | Item descriptions | FF Factor1 | FF Factor2 |
|---|---|---|---|
| FF1 | Muscle pain | **0.455** | 0.091 |
| FF2 | Muscle tension | **0.630** | 0.282 |
| FF3 | Fatigue | **0.776** | 0.146 |
| FF4 | Concentration Disorders | 0.144 | **0.965** |
| FF5 | Memory disturbances | 0.057 | **0.981** |
| FF6 | Irritability | 0.309 | **0.401** |
| FF7 | Sadness | **0.741** | 0.236 |
| FF8 | Sleep disorders | **0.580** | 0.115 |
| FF10 | Gastro-intestinal symptoms | **0.572** | 0.112 |
| FF11 | Headache | **0.450** | 0.053 |
| FF12 | Flu-like malaise | 0.243 | -0.127 |
|  |  |  |  |
|  | Variance | 2.791 | 2.266 |
|  | ORION marginal reliability | 0.840 | 0.978 |
|  | Factor determinacy index | 0.916 | 0.989 |
|  | Generalized H index | 0.845 | 0.987 |
|  | Expected percentage of true differences | 90.0% | 97.6% |

Shown are the loadings on the weighted varimax-rotated factors. Highly loaded items (>0.4) are shown in bold and are underlined.

ORION: Overall Reliability of Fully Informative prior Oblique N-EAP scores

Table 4. Intercorrelation matrix of the Fibromyalgia and Chronic Fatigue Syndrome (FF) Rating Scale FF symptoms, dialysis features and biomarkers in subjects with end-stage renal disease (ESRD).

| Biomarkers | FF total score | FF Factor1 items | FF Factor2 items |
|---|---|---|---|
| Duration of ESRD[#] | 0.242 | 0.262* | 0.136 |
| Number dialysis sessions per week[#] | 0.422** | 0.396** | 0.431** |
| Total number of dialysis sessions[#] | 0.269* | 0.283* | 0.159 |
| Estimated glomerular filtration rate | -0.535** | -0.521** | -0.022 |
| Urea | 0.598** | 0.575** | 0.011 |
| Creatinine | 0.531** | 0.513** | 0.005 |
| Hemoglobin | -0.526** | -0.532** | -0.004 |
| Albumin | -0.334** | -0.308** | -0.101 |
| Phosphate | 0.356** | 0.332** | -0.049 |
| Zinc | -0.270** | -0.245* | -0.097 |
| Copper | 0.274** | 0.302** | 0.136 |
| Cations / chloride ratio | 0.104 | 0.094 | 0.425** |
| Dickkopf protein 1 | 0.112 | 0.151 | -0.112 |
| R-Spondin-1 | 0.028 | 0.039 | -0.182 |
| Sclerostin | 0.036 | 0.097 | -0.192 |
| β-Catenin | -0.356** | -0.319** | -0.080 |

*$p<0.05$, **$p<0.01$ (all n=90). All results of Spearman's rank order coefficients. [#] Performed in ESRD patients; other analyses: performed in patients and controls combined.

Table 5. Results of multiple regression analysis with the total score on the Fibromyalgia and Chronic Fatigue Syndrome (FF) Rating Scale as dependent variable and Wnt-pathway proteins and eGFR related biomarkers as explanatory variables.

| Regression | Explanatory variables | β | t | p | $F_{model}$ | df | p | $R^2$ |
|---|---|---|---|---|---|---|---|---|
| **#1.** FF total score in the total study group | **Model** | | | | 26.66 | 2/86 | <0.001 | 0.383 |
| | eGFR | -0.617 | -7.20 | <0.001 | | | | |
| | Cations / Chloride | 0.200 | 2.33 | 0.022 | | | | |
| **#2.** FF total score in the total study group | **Model** | | | | 32.07 | 2/86 | <0.001 | 0.427 |
| | Urea | 0.659 | 7.90 | <0.001 | | | | |
| | Cations / Chloride | 0.239 | 2.87 | 0.005 | | | | |
| **#3.** FF total score in the total study group | **Model** | | | | 13.71 | 4/84 | <0.001 | 0.395 |
| | Hemoglobin | -0.418 | -4.73 | <0.001 | | | | |
| | β-Catenin | -0.459 | -3.67 | <0.001 | | | | |
| | R-spodin 1 | 0.346 | 2.80 | 0.006 | | | | |
| | Age | 0.218 | 2.53 | 0.013 | | | | |
| **#4.** FF total score in patients only | **Model** | | | | 9.38 | 3/55 | <0.001 | 0.338 |
| | Dialysis session/ Week | 0.481 | 4.32 | <0.001 | | | | |
| | R-spodin 1 | 0.506 | 3.31 | 0.002 | | | | |
| | β-Catenin | -0.375 | -2.46 | 0.017 | | | | |

eGFR: estimated glomerular filtration rate.

Table 6. Results of multiple regression analysis with two subdomains scores of the Fibromyalgia and Chronic Fatigue Syndrome (FF) Rating Scale as dependent variable and Wnt-pathway proteins and end-stage renal disease related biomarkers as explanatory variables.

| Regressions | Explanatory variables | β | t | p | F $_{model}$ | df | p | R$^2$ |
|---|---|---|---|---|---|---|---|---|
| #1. Sum 7 FF Factor 1 items | **Model** | | | | 61.84 | 1/87 | <0.001 | 0.415 |
| | Urea | 0.645 | 7.86 | <0.001 | | | | |
| #2. Sum 7 FF Factor 1 items | **Model** | | | | 56.00 | 1/87 | <0.001 | 0.392 |
| | eGFR | -0.626 | -7.48 | <0.001 | | | | |
| #3. Sum 7 FF Factor 1 items | **Model** | | | | 15.00 | 4/84 | <0.001 | 0.417 |
| | Hemoglobin | -0.459 | -5.29 | <0.001 | | | | |
| | β-catenin | -0.433 | -3.53 | 0.001 | | | | |
| | R-Spondin 1 | 0.296 | 2.44 | 0.017 | | | | |
| | Age | 0.200 | 2.36 | 0.020 | | | | |
| #4. Sum 3 FF Factor 2 items | **Model** | | | | 8.79 | 4/84 | <0.001 | 0.295 |
| | Cations / Chloride ratio | 0.419 | 4.48 | <0.001 | | | | |
| | Sex | 0.220 | 2.35 | 0.021 | | | | |
| | Body mass index | -0.241 | -2.59 | 0.011 | | | | |
| | Age | 0.192 | 2.07 | 0.041 | | | | |

eGFR: estimated glomerular filtration rate.
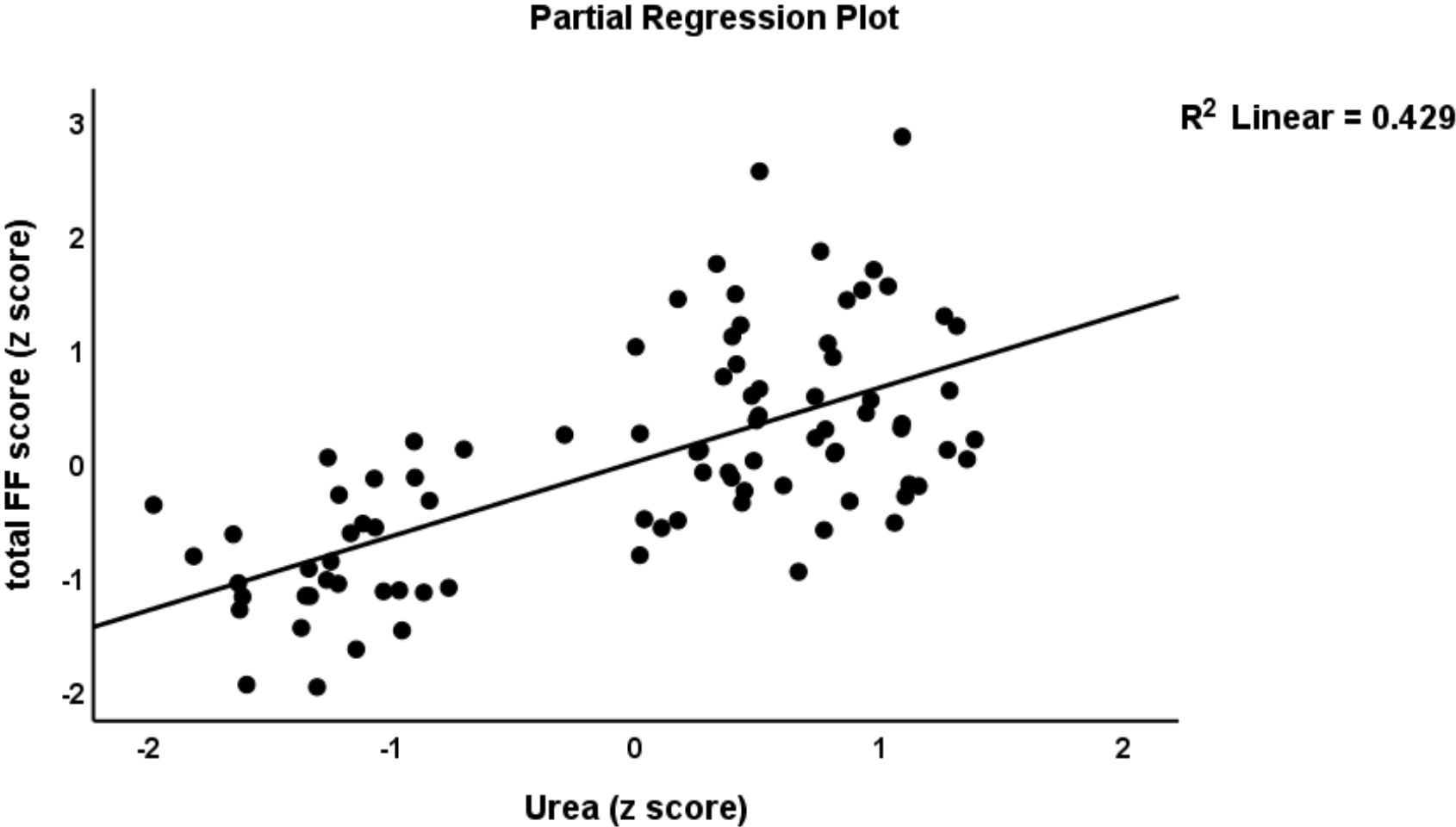
Figure 1. Partial regression of the total Fibromyalgia and Chronic Fatigue Syndrome (FF) Rating Scale score on urea.
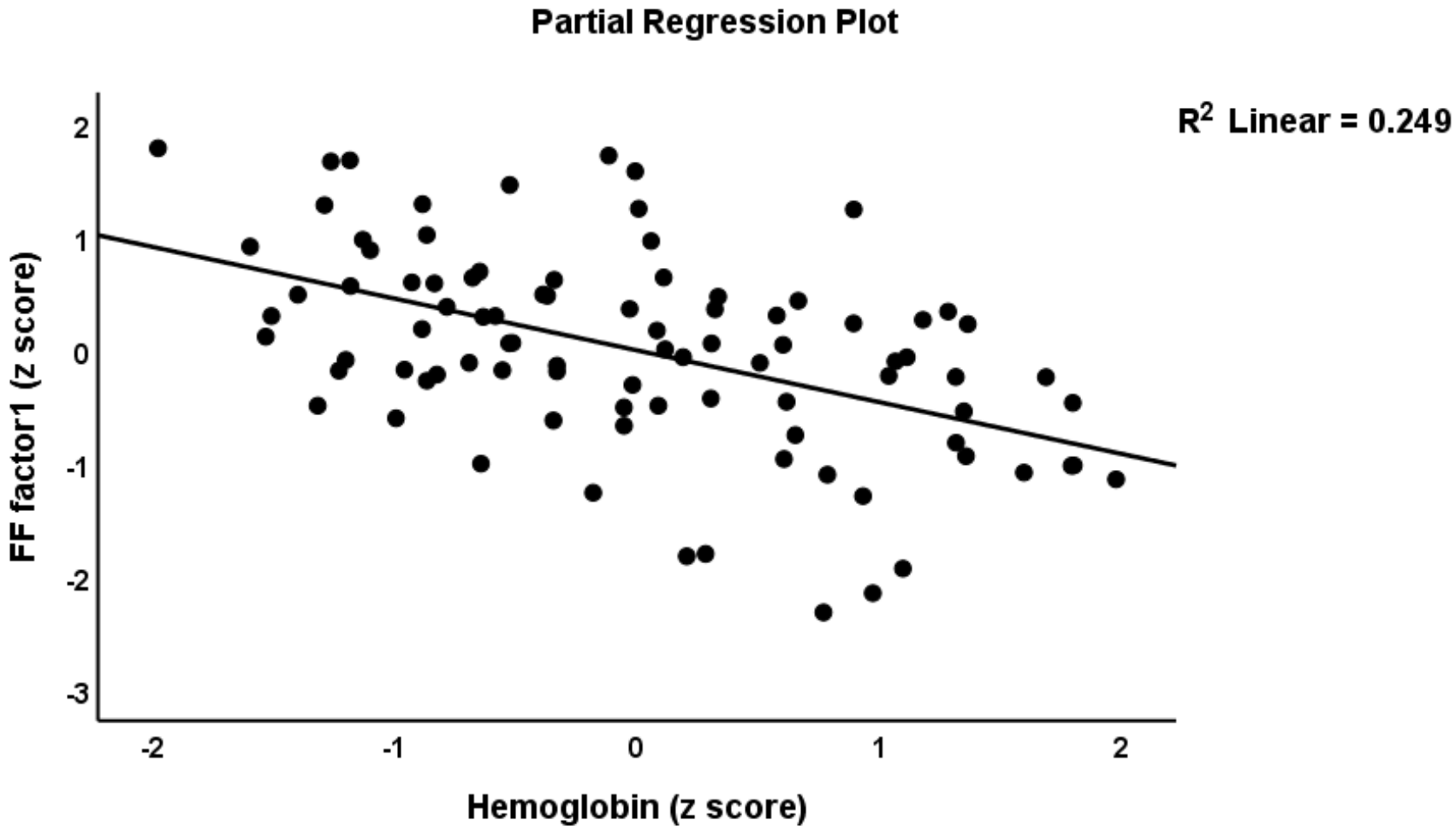
Figure 2. Partial regression of the first factor extracted from the items of the Fibromyalgia and Chronic Fatigue Syndrome (FF) Rating Scale score on hemoglobin.
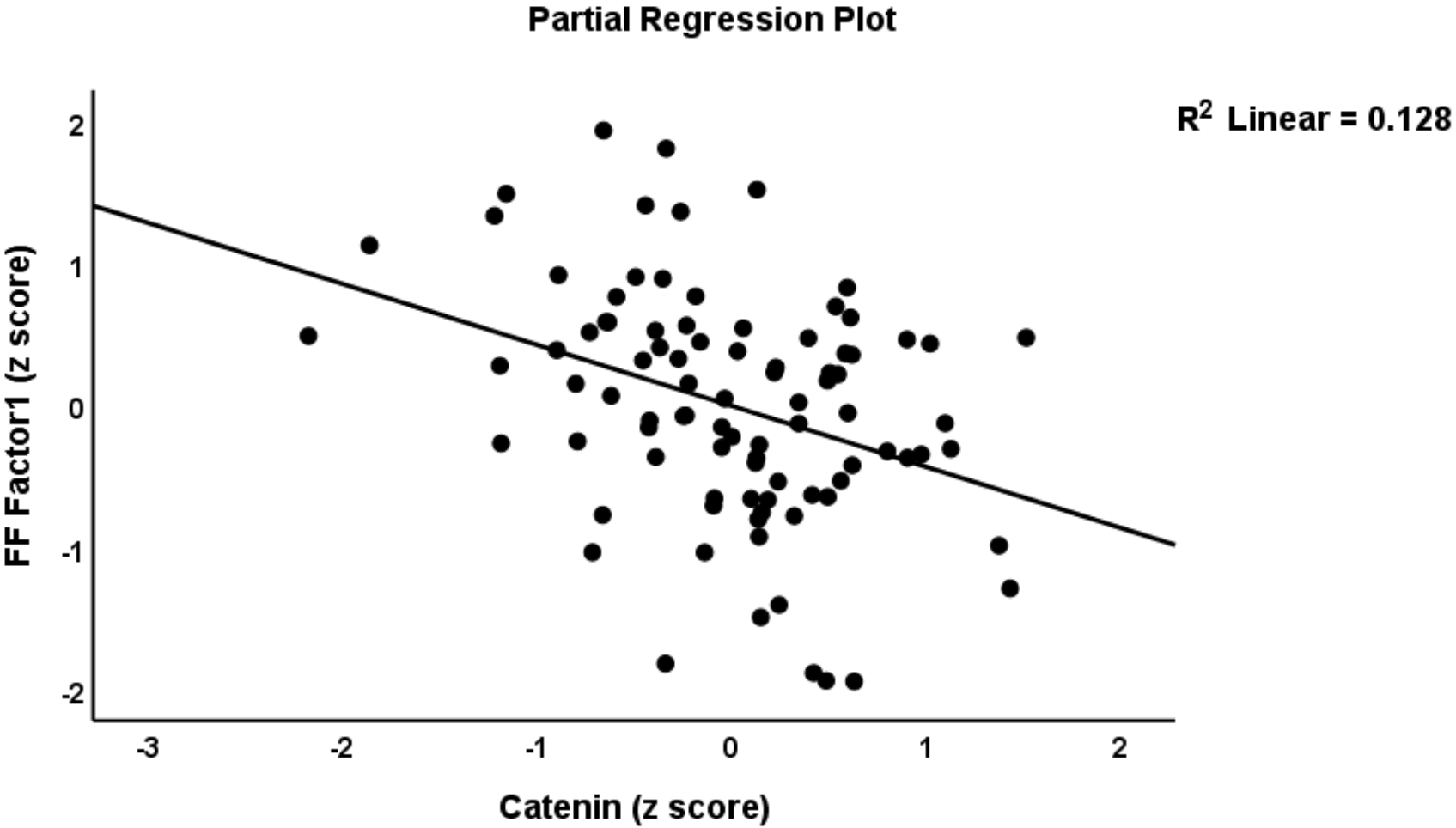
Figure 3. Partial regression of the first factor extracted from the items of the Fibromyalgia and Chronic Fatigue Syndrome (FF) Rating Scale score on β-catenin.
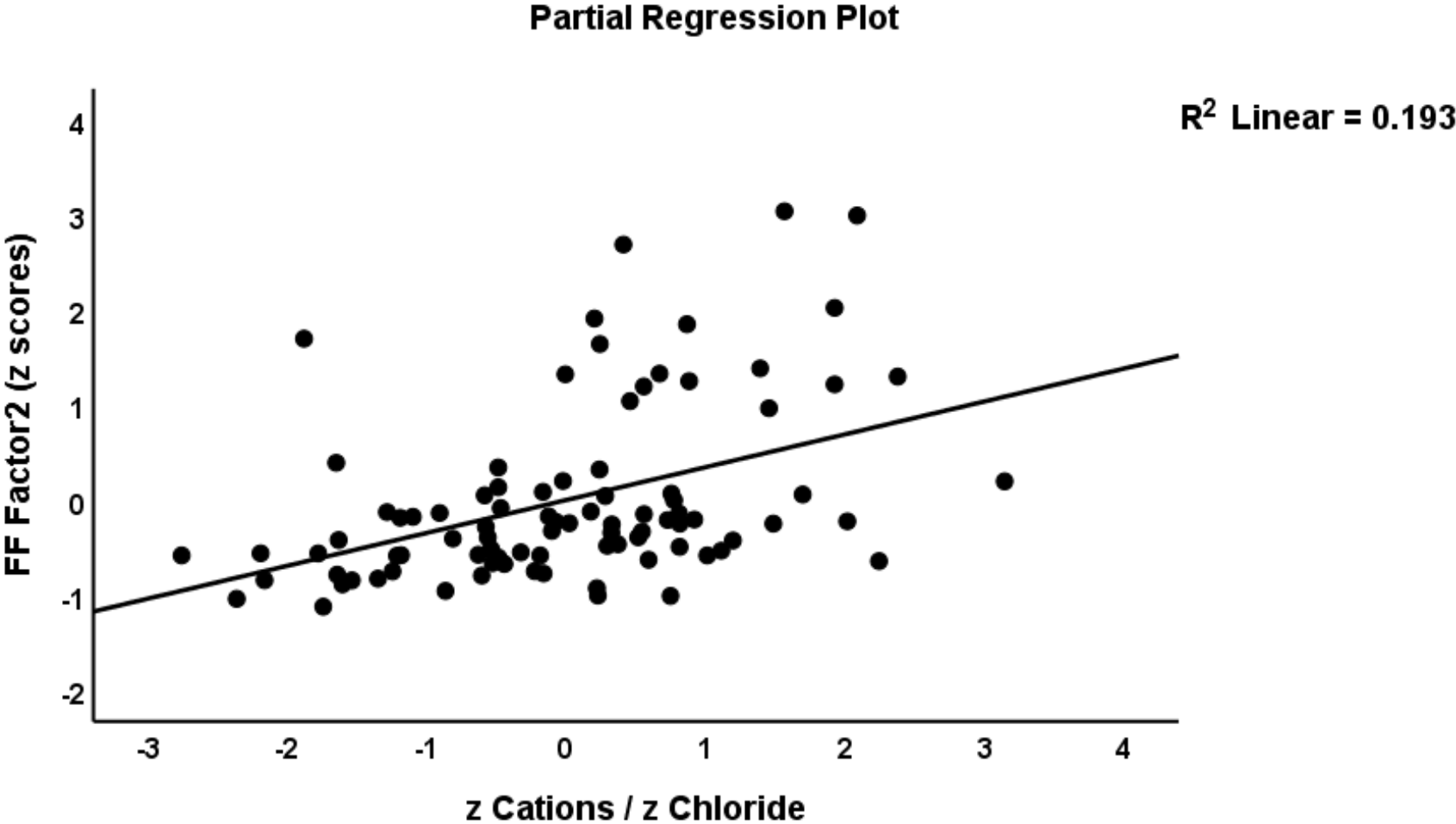
Figure 4. Partial regression of the second factor extracted from the items of the Fibromyalgia and Chronic Fatigue Syndrome (FF) Rating Scale score on the Cations / Chloride ratio.
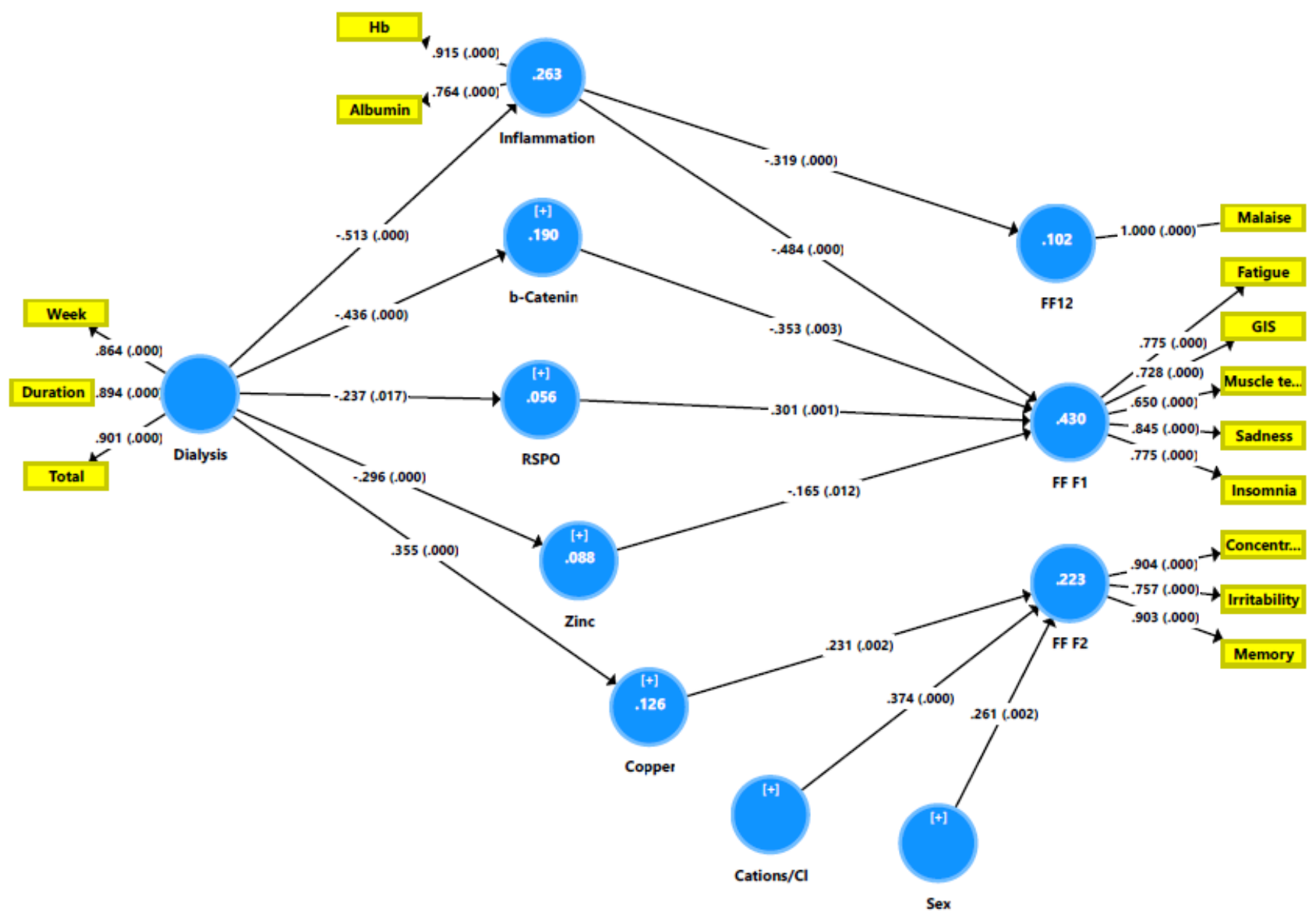
Figure 5. Results of Partial Least Squares analysis.

FF F1/FF F 2: first and second factor extracted from the items of the Fibromyalgia and Chronic Fatigue Syndrome (FF) Rating Scale. F1 symptoms are fatigue, gastro-intestinal symptoms (GIS), muscle tension (muscle te), sadness, and insomnia. F2 symptoms are concentration (concentr) and memory impairments, and irritability. FF12: item 12 of the FF scale (a flu-like malaise). Inflammation: a latent vector extracted from hemoglobin (Hb) and albumin. RSPO: R-spondin 1. Cations/Cl: ratio of cations on chloride. Dialysis: a latent vector extracted from duration of illness and total and weekly numbers of hemodialysis sessions. White figures in the blue circles denote explained variance.