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Glutathione antioxidant mechanism in haemodialysis patients after 1 year: observational and longitudinal study

Mecanismo antioxidante da glutationa em pacientes em hemodiálise após 1 ano: estudo observacional longitudinal

Mecanismo antioxidante del glutatión en pacientes en hemodiálisis después de 1 año: estudio observacional longitudinal

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ABSTRACT

Objective: To analyze the variation in oxidative stress (OS) biomarkers, focusing on the glutathione mechanism, after a one-year period in the same patients on haemodialysis. **Methods:** Observational and longitudinal study performed in a dialysis clinic at two moments. The following biomarkers of the glutathione mechanism were analysed in the same individuals at baseline and after a one-year period: reduced glutathione (GSH), oxidized glutathione (GSSG), the GSH:GSSG ratio, glutathione peroxidase (GPx). For statistical analysis, the paired Student's t-test, Wilcoxon test, Chi-square test, and Pearson's or Spearman's correlation were adopted. Effect sizes were calculated with Hedges' g and Cliff's delta. For the retrospective power analysis G*Power® software was used. P <0.05 was considered statistically significant. **Results:** Ten patients concluded the study. After a one-year period, an increase was observed in GSSG (P=0.000), GPx (P=0.041), and a decrease in GSH (P=0.007) and the GSH:GSSG ratio (P=0.000). Moderate positive not significant correlations were found between HD vintage and GSSG (rho = 0.4108, p=0.5), and a moderate negative correlation between GSH (rho = -0.5817, p=0.007) and the GSH:GSSG ratio (rho = -0.5123, p=0.025). **Conclusion:** There was a significant increase in OS related to glutathione mechanism.

Keywords: Renal dialysis, Oxidative stress, Glutathione, Reactive oxygen species, Nutritional assessment.

RESUMO

Objetivo: Analisar biomarcadores de estresse oxidativo (EO), com foco no mecanismo da glutationa, após um período de um ano nos mesmos pacientes em hemodiálise. **Métodos:** Estudo observacional e longitudinal realizado em uma clínica de diálise. Foram analisadas: glutationa reduzida (GSH), glutationa oxidada (GSSG), a razão GSH:GSSG e glutationa peroxidase (GPx). Para a análise estatística, foram utilizados o teste t de Student pareado, o teste de Wilcoxon, o teste do qui-quadrado e a correlação de Pearson ou Spearman. Os tamanhos do efeito foram calculados com o g de Hedges e o delta de Cliff. Para a análise retrospectiva do poder estatístico, foi utilizado o software G*Power®. P < 0,05 foi considerado estatisticamente significativo.

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Resultados: Dez pacientes concluíram o estudo. Após um período de um ano, observou-se aumento nos níveis de GSSG (P=0,000) e GPx (P=0,041), e diminuição nos níveis de GSH (P=0,007) e na razão GSH:GSSG (P=0,000). Correlações positivas moderadas, não significativas, foram encontradas entre o tempo em hemodiálise e os níveis de GSSG (rho = 0,4108, p=0,5), e uma correlação negativa moderada entre os níveis de GSH (rho = -0,5817, p=0,007) e a razão GSH:GSSG (rho = -0,5123, p=0,025). **Conclusão:** Houve aumento significativo no EO relacionado ao mecanismo da glutationa.

Palavras-chave: Diálise renal, Estresse oxidativo, Glutationa, Espécies reativas de oxigênio, Avaliação nutricional.

RESUMEN

Objetivo: Analizar biomarcadores de estrés oxidativo (EO), centrándose en el mecanismo del glutatión, después de un año en los mismos pacientes en hemodiálisis. **Métodos:** Estudio observacional y longitudinal realizado en una clínica de diálisis. Se analizaron glutatión reducido (GSH), glutatión oxidado (GSSG), cociente GSH:GSSG y glutatión peroxidasa (GPx). Para análisis, se utilizaron pruebas t de Student para muestras pareadas, prueba de Wilcoxon, prueba de chi-cuadrado y correlación de Pearson o Spearman. Los tamaños del efecto se calcularon mediante la g de Hedges y el delta de Cliff. Para el análisis retrospectivo de la potencia estadística, se utilizó el software G*Power®. P < 0,05 se consideró estadísticamente significativo. **Resultados:** Diez pacientes completaron el estudio. Tras un año, se observó aumento de GSSG (P = 0,000) y GPx (P = 0,041), así como disminución de GSH (p = 0,007) y del cociente GSH:GSSG (P = 0,000). Se encontraron correlaciones positivas moderadas, no significativas, entre tiempo en hemodiálisis y niveles de GSSG (rho = 0,4108, p = 0,5), y una correlación negativa moderada entre GSH (rho = -0,5817, p = 0,007) y el cociente GSH:GSSG (rho = -0,5123, p = 0,025). **Conclusion:** Hubo aumento significativo de EO relacionado con el mecanismo del glutatión.

Palabras clave: Diálisis renal, Estrés oxidativo, Glutatión, Especies reactivas de oxígeno, Evaluación nutricional.

INTRODUCTION

Chronic kidney disease (CKD) is characterized by progressive and irreversible loss of kidney function, and with the progression of the disease, renal replacement therapy, such as haemodialysis (HD), is required to maintain patient survival (STEVENS PE, et al., 2024). Oxidative stress (OS) and inflammation act synergically, and it is difficult to discern which process is primarily responsible for initiating the series of events that eventually lead to CKD (LIAKOPOULOS V, et al., 2019).

The pathophysiology of increased OS is multifactorial and is aggravated by decreased antioxidant intake due to dietary restrictions, the use of diuretics, compromised nutritional status, bioincompatible membranes, and loss of antioxidants during the HD process (LIAKOPOULOS V, et al., 2019; LIAKOPOULOS V, et al., 2017; MORENA M, et al., 2002).

Oxidative stress, resulting from an imbalance between pro and antioxidative mechanisms, is a recognized phenomenon in HD patients, that contributes to long-term complications (MORENA M, et al., 2002). HD patients tend to present altered levels of antioxidant enzymes and, in general, an increase in OS markers, especially in more advanced stages of CKD (LIAKOPOULOS V, et al., 2019; DAENEN K, et al., 2019). Reduced glutathione (GSH) is the most abundant non-protein thiol antioxidant in mammalian tissues and is directly impacted by changes in OS (YEH E-L et al., 2020).

Alterations in the GSH antioxidant system, including decreased blood levels of GSH and increased GSSG and erythrocyte GPx have been reported in CKD patients, highlighting the potential of GPx to modulate OS by selective positive regulation of several selenoproteins involved in antioxidant defense (TOUAT-HAMIC Z et al., 2014), as well as being associated with increased reactive oxygen species (ROS) (TOUAT-HAMIC Z, et al., 2014; HENSLEY K, et al., 2004).

Diepeveen SHA et al. (2005), in a prospective cohort study, assessed patients with end-stage renal disease prior to the start of hemodialysis and after 6 months of treatment. Regarding lipid peroxides and in vitro copper-induced LDL, no changes in oxidation were observed. Another prospective cohort study did not show significant alterations in plasma protein carbonyl content after the patients started HD, and at the end of the one-year study period (PUPIM LB, et al., 2004).

Although several studies have evaluated oxidative stress at a specific time (comparison) or after mineral and/or vitamin supplementation, we did not find studies that evaluated the response of the glutathione mechanism in the same patients before and after a one-year period.

Our hypothesis was that patients in maintenance HD would present worsening in the antioxidant mechanism after one year. Thus, the aim of the present study was to evaluate the variation in OS biomarkers, focusing on the glutathione mechanism, after a one-year period, in patients with CKD on HD. The secondary aim was to evaluate malondialdehyde (MDA) in the same patients.

METHODS

An observational and longitudinal study was carried out with patients from a HD clinic. The sampling was based on temporal saturation and data collection was performed in April 2019 and April 2020. The sample was non-probabilistic by convenience. Inclusion criteria were age ≥ 19 years, undergoing HD two or three times a week, and signing the consent form to participate in the study. Exclusion criteria were: performing dialysis for less than three months; use of immunosuppressants; infection with the human immunodeficiency virus; cancer, advanced heart failure, chronic lung disease, or liver disease; hospitalization; and the inability to understand or communicate.

The patients were informed about the objectives and procedures of the study and provided written informed consent prior to data collection. The study was approved by the Human Ethics Committee at the University (CAAE: 76599817.6.0000.0121; Approval No.: 3.553.845).

Results of routinely assessed blood parameters conducted by the dialysis clinics, including serum albumin, Kt/V, serum phosphorus, and potassium levels were obtained from patient medical records. Blood samples were collected at baseline and at the one-year follow-up, after the second HD session of the week. The collection was performed via vascular access (fistula or catheter) into four tubes: one containing heparin (4 mL), two containing ethylenediaminetetraacetic acid (EDTA) (4 mL each), and one containing a clot activator (9 mL).

The blood samples were transported in an ice-cold container (up to 1 hour after collection). Three of the four blood tubes (one with EDTA, one with a clot activator, and one with heparin) were centrifuged (5000 RPM, 10 minutes, temperature 4°C) to obtain plasma and serum.

For GSH and GSSG analyses, whole blood was divided into aliquots in microtubes containing 100 µL of 310 mM N-ethylmaleimide (NEM) per mL/blood. Hemolysate was used for GPx determination, from 100 µL of red blood cells and 1.0 mL of hemolysing solution (4nM MGSO4 4nM acetic acid solution). All samples were stored in triplicate at -80 °C until the time of analysis.

Oxidative stress was measured through reduced glutathione (GSH), oxidized glutathione (GSSG), the GSH:GSSG ratio, glutathione peroxidase (GPx), and malondialdehyde (MDA). The OS biomarkers were analysed according to a previous study conducted by this research group (MARTINS ML, et al., 2021), including MDA (DOMIJAN A-M, et al., 2015; GROTTO D, et al., 2007), GSH, GSSG (GIUSTARINI D, et al., 2013), and GPx (WENDEL A, 1981). The GSH:GSSG ratio was calculated using the formula: GSH (µmol / g Hb) / GSSG (nmol / g Hb). The sample was evaluated at baseline and after one year using indicators of nutritional and functional status.

Body weight was measured immediately after the hemodialysis session to minimize fluid overload bias, and the assessments were performed within 30 minutes post-dialysis, on the second HD weekday. The body mass index (BMI) was calculated.

The 7-point Subjective Global Assessment (7p-SGA) was applied according to recommendations for dialysis patients (IKIZLER TA, et al., 2020); individuals scoring ≥6 were classified as well-nourished, and those with scores <6 as malnourished (CANUSA, 1996).

Malnutrition was also diagnosed according to the Global leadership initiative on malnutrition (GLIM) criteria, following the approach proposed, which requires the presence of at least one phenotypic criterion and one etiologic criterion after initial nutritional risk screening (CEDERHOLM T, et al., 2019). The functional indicator handgrip strength (HGS) (GARCIA MF, et al., 2013) was evaluated with a Saehan® model SH 5001 hydraulic hand dynamometer (Saehan Corporation - Yangdeok-Dong, Masan, Korea).

Data were analysed using Stata 13, Data Analysis and Statistical Software (Stata Corporation, College Station, TX, USA). The normality of data distribution was assessed by the Shapiro-Wilk test; asymmetric variables (P≤0.05) are presented as median and interquartile range and symmetric variables (P>0.05) as mean and standard deviation. The logarithmic transformation of the data was performed (GPx, GSSG, GSH:GSSG ratio). Differences in means at baseline and after one year were assessed using the paired Student's t test and the Wilcoxon test, and qualitative variables were assessed using the Chi-square test.

Pearson's or Spearman's correlation was performed to evaluate the correlation between HD vintage and OS biomarkers. The correlation was considered weak when the value was from 0 to 0.29, moderate from 0.30 to 0.69, and strong from 0.70 to 1.0 (ARANGO HG, 2009).

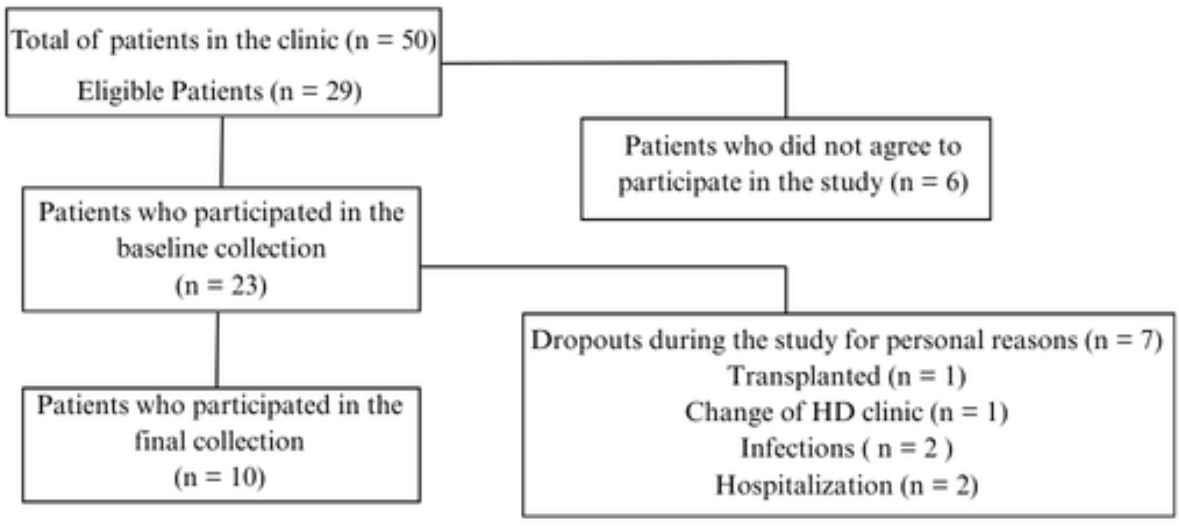
Effect size was calculated using Hedges' g statistic (g), which represents the Cohen's effect size after appropriate correction for symmetric variables (LAKENS D, 2013; COHEN J, 1992). The effect size g was interpreted as: trivial (<0.20), small (0.20 to 0.49), medium (0.50 to 0.79), and large (>0.80) (COHEN J, 1992). For the asymmetric variables, the effect size was Cliff's delta (δ) which was classified as: negligible (<0.147), small (0.148 - 0.32), medium (0.33 - 0.474), and strong (>0.474) (LAKENS D, 2013).

Retrospective power (1 – b) calculation for the OS outcomes (MDA, GSH, GSSG, GSH:GSSG ratio, and GPx) with n=10 was carried out using G*Power®. A value of P≤0.050 was adopted as statistically significant.

RESULTS

Ten patients completed the study, the mean age of the participants at the beginning of the study was 53 ± 14.13 years, with an HD vintage of 3.30±0.67 years. Most of the patients performed HD twice a week (n=6), were female (n=6), and hypertensive (n=8), and 50% of the sample had diabetes type 2 (n=5).

Figure 1 - Flowchart of the study.



Source: Machado RP, et al., 2026.

Table 1 - Comparison of biochemical, functional and nutritional parameters at baseline and after a 1 year-follow-up in haemodialysis patients.

	Baseline (N=10)	After 1 year (N=10)	P
Dialysis adequacy (Kt/V)¥	1.31±0.27	1.29±0.23	0.769 [a]
Phosphorus (mg/dL)¥	5.88±1.04	5.83±1.52	0.930 [a]
Potassium (mEq/L)¥	5.02±0.94	5.45±1.15	0.327[a]
Albumin (g/dL)¥	3.24±0.23	3.55±0.21	0.005[a]
BMI (kg/m²)¥	28.70±6.36	28.82±5.75	0.862[a]
HGS (kg)¥	32.11±14.61	28.88±14.89	0.064 [a]
7p-SGA			0.414[b]
Well-nourished	2 (20%)	4 (40%)	
Malnutrition	8 (80%)	6 (60%)	
GLIM			0.157 [b]
Well-nourished	3 (30%)	5 (50%)	
Malnutrition	7 (70%)	5 (50%)	

Values for categorical variables are given as number (percentage), median and interquartile range (IQ25-75) (§) and mean and standard deviation (¥). Statistical analysis: [a]Paired T-test; [b]Wilcoxon test.
Abbreviations: HD: haemodialysis; BMI: body mass index; 7p-SGA: Seven-point subjective global assessment; GLIM: global leadership initiative on malnutrition; HGS: Handgrip strength.
Source: Machado RP, et al., 2026.

In a period of one year, 2 patients (20%) became nourished (not statistically significant), according to the 7p-SGA (p = 0,414), and GLIM (p = 0,157).

Table 2 - Oxidative stress biomarkers at baseline and after a 1-year-follow-up in haemodialysis patients.

Biomarker	Basal (N=10)	After 1 year (N=10)	P	Effect size
MDA (µmol/L)¥	0.82±0.11	4.15±0.37	0,000[a]	1 (-0.507 – 0.433)[d]
GSH (µmol/g Hb)§	14.23 (13.17 – 15.24)	6.97 (6.06 – 7.27)	0.007[b]	-0.90 (-0.985 – -0.455)[d]
GSSG (nmol/g Hb)¥	52.92±45.55	162.25±76.95	0,000[a]*	-1.52 (-2.646 – -0.658)[c]
GSH/GSSG ratio¥	0.45±0.27	0.05±0.03	0.000[a]*	- 1 (-0.507 – 0.433)[d]
GPx (U/mg Hb)¥	11.67±4.86	27.33±23.92	0.041[a]*	0.8161 (-1.711 – -0.036)[c]

Values for categorical variables are given as number (percentage), median and interquartile range (IQ25-75) (§) and mean and standard deviation (¥). Statistical analysis: [a]Paired T-test; [b]Wilcoxon test, [c]Cohen's delta (IQ95%); [d]Cliff's delta (IQ95%). *Data transformed to logarithm.
Abbreviations: MDA: malondialdehyde; GSH: reduced glutathione; GSSG: oxidized glutathione; GPx: glutathione peroxidase.
Source: Machado RP, et al., 2026.

Moderate positive not significant correlations were found between HD vintage and MDA (rho = 0.4421, p=0.051) and GSSG (rho = 0.411, p=0.5) and a moderate negative correlation between GSH (rho = -0.5817, p=0.007) and the GSH:GSSG ratio (rho = -0.5123, p=0.025). The retrospective power analysis (1 - b) indicated a power of 0.98 for MDA, 0.97 for GSH, 0.99 for GSSG and the GSH:GSSG ratio, and 0.93 for GPx.

DISCUSSION

The kidneys play a central role in the production of endogenous antioxidant enzymes. As a result, renal impairment is often accompanied by a reduction in these enzymes and an increase in pro-oxidant molecules. Moreover, the accumulation of uremic toxins contributes significantly to the development and persistence of OS in patients with CKD (RYSZ J, et al., 2020).

The glutathione system plays a pivotal role in maintaining cellular redox balance (ZHENG C-M, et al., 2024). Worsening of the antioxidant mechanism was previously recognized in patients with CKD on HD (LIAKOPOULOS V, et al., 2019) and contributes to progressive impairment in kidney function and higher morbidity and mortality rates (LIAKOPOULOS V, et al., 2017).

Alterations in the glutathione antioxidant system, including reduced GSH levels alongside increased GSSG and GPx, have been documented in CKD patients, with variations reported among studies (ZHENG C-M, et al., 2024; RAPA SF, et al., 2020). Elevated GPx activity, while initially protective, may paradoxically be associated with adverse outcomes in dialysis patients, possibly reflecting an underlying redox imbalance rather than an effective defense (MORENA M, et al., 2002). Additionally, the inflammatory state linked to OS is highlighted by elevated markers, such as GSSG and the GSH:GSSG ratio, combined with decreased GSH levels (POULIANITI KP, et al., 2016).

Although some studies have focused on oxidative stress in haemodialysis patients, to our knowledge, this is the first study to compare the glutathione system involved in OS in the same HD patients after a one-year period.

Even though the evolution of CKD contributes to an increase in OS (POULIANITI KP, et al., 2016) and a decrease in antioxidant factors (LIAKOPOULOS V, et al., 2019), heterogeneous results have been reported regarding antioxidant capacity in HD patients (POULIANITI KP, et al., 2016). Erythrocyte GSH levels decreased, increased (ROEHRS M, et al., 2011; VALENTINI J, et al., 2008) or presented similar values (KHAZIM K, et al., 2013; IBRAHIM VH, et al., 2010) in HD patients when compared to healthy subjects. This discrepancy in results between studies may be due to different dialysis vintages and the heterogeneity of individuals (YEH E-L, et al., 2020). The discrepancy may also be caused by the lack of standardization of analytical methods, specifically regarding spontaneous oxidation of GSH after analyte collection (KHAZIM K, et al., 2013).

Despite heterogeneous results, evidence suggests that both GSH and GSSG are significantly utilized during a single HD session, and changes in their levels are associated with increased OS (YEH E-L, et al., 2020). In our study, we observed a significant decrease in GSH levels and in the GSH:GSSG ratio after one year, which reflects a progressive redox imbalance in these patients. These findings are consistent with previous reports showing GSH depletion in this population (YEH E-L, et al., 2020).

Patients undergoing haemodialysis may have higher demands for GSH to cope with elevated OS generated during the procedure (YEH E-L, et al., 2020). To obtain a more reliable measure of the cellular redox state, the GSH:GSSG ratio has been recommended (KHAZIM K, et al., 2013). The GSH:GSSG ratio has been proposed as a reliable indicator of OS in patients with CKD, given the characteristic reduction in GSH and/or accumulation of GSSG observed in this population (YEH E-L, et al., 2020).

As mentioned earlier, two follow-up studies with CKD patients analyzed OS over a period of 6 months (DIAPEVEEN SHA, et al., 2005) or one year of dialysis treatment (PUPIM LB, et al., 2004). The authors did not demonstrate significant increases in plasma lipid peroxides, in vitro copper-induced LDL (DIAPEVEEN SHA, et al., 2005), or protein carbonyl content (PUPIM LB, et al., 2004).

A previous study observed that erythrocyte MDA levels were higher in HD patients compared with healthy controls and patients under conservative treatment (without dialysis), suggesting a significant increase in OS in HD patients compared with the other two groups ($p < 0.005$) (DURAK I, et al., 2004).

A Study by Koca T, et al. (2010), evaluated plasma levels of OS in HD patients (HD vintage: 0 to 2 years, 3 to 5 years, 6 to 8 years, and 9 to 11 years) and healthy controls, and found that plasma levels of MDA were significantly higher in HD patients than in healthy controls ($P<0.01$). In addition, MDA levels were higher in the groups with a longer dialysis time (6 to 11 years) than those on HD treatment for shorter periods (0 to 5 years) ($p < 0.050$). In line with these findings, our study also identified a significant increase in MDA concentration after one year ($p = 0.000$), reinforcing the evidence that lipid peroxidation is progressively elevated in HD patients.

Valentini J, et al. (2008), compared two groups of HD patients on treatment for 17.7 months and for 82.2 months, as well as a healthy control group. Among the results, the authors found that patients with longer HD treatment presented higher MDA values than patients on HD for less time and healthy controls. In relation to lipid peroxidation, we observed significant increase in MDA concentration, suggesting a progression of OS after one-year period, in the same HD patients. Likewise, the positive correlation (not significant) between HD vintage and MDA (rho = 0.4421; p = 0.051) further suggests that longer exposure to dialysis may contribute to greater oxidative damage. In addition, a moderate negative correlation between HD vintage and GSH concentrations and the GSH:GSSG ratio.

Although not yet fully elucidated, the pathophysiological mechanisms of OS and inflammation are interrelated and synergistic in CKD (LIAKOPOULOS V, et al., 2019). This interplay is further exacerbated by the inadequate clearance of uremic toxins, which accumulate as renal function declines and exert detrimental effects on cellular function (RYSZ J, et al., 2020).

OS has the capacity to activate immune cells such as macrophages, neutrophils, and monocytes, promoting the release of pro-inflammatory mediators. These activated cells can produce ROS as part of the innate immune response. On the other hand, ROS can enhance the synthesis of pro-inflammatory cytokines. This reciprocal interaction establishes a positive feedback loop between OS stress and inflammation, which may sustain chronic inflammatory states and contribute to progressive tissue damage in multiple organs. Moreover, many risk factors associated with oxidative stress also overlap with those involved in systemic inflammation (CHANDA D, et al., 2023).

In the present study, after a one-year period, the studied patients showed significant increases in MDA, GSSG, and GPx, and decreases in GSH concentrations and the GSH:GSSG ratio. This increase in GPx activity after one year may reflect a compensatory mechanism in response to sustained OS. This enzymatic upregulation has been previously reported in HD patients as part of an adaptive response to elevated levels of ROS (LIAKOPOULOS V, et al., 2017). However, despite this apparent compensation, the simultaneous increase in GSSG and MDA, along with the reduction in GSH, suggests that the antioxidant system remains functionally overwhelmed. Similar findings have shown increased GPx activity occurring in parallel with markers of lipid peroxidation in dialysis patients, suggesting that GPx elevation may not be sufficient to restore redox homeostasis in the long term (MORENA M et al., 2002). These results reinforce the importance of monitoring both enzymatic and non-enzymatic components of the antioxidant defense in CKD patients undergoing HD.

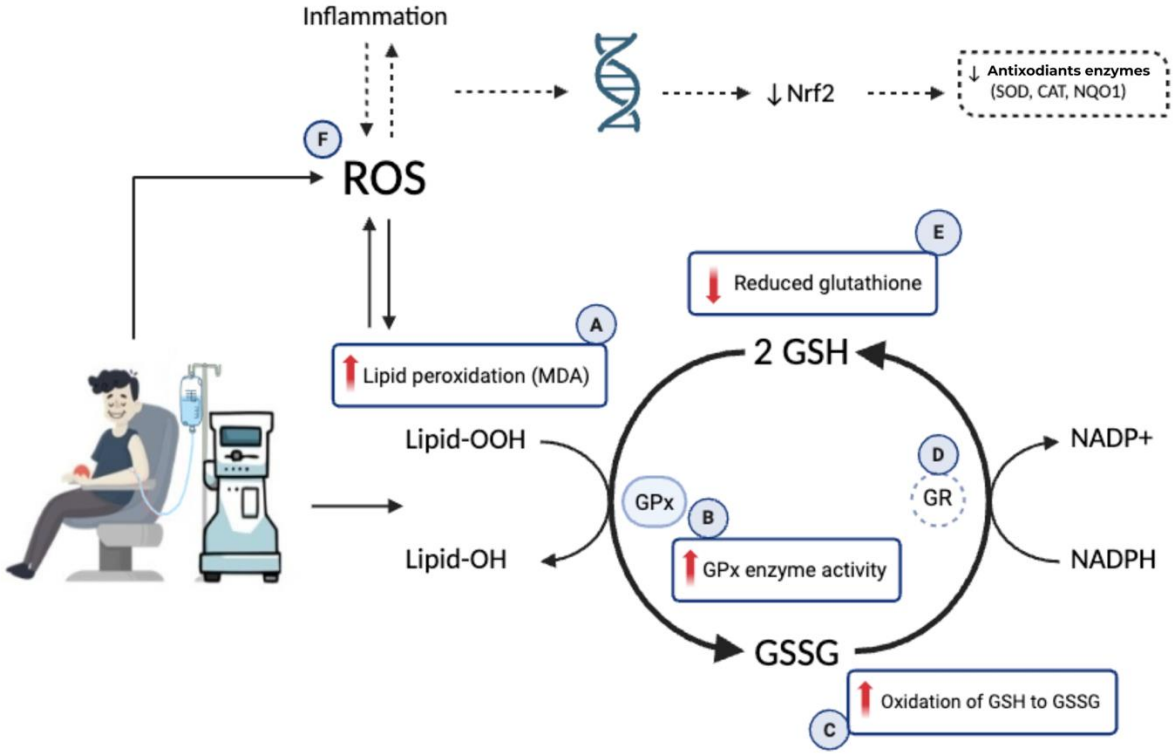
The concentration of GSH is controlled by the rate of oxidation, conjugation, extrusion, uptake of thiol-containing precursors, and resynthesis (URSINI F and MAIORINO M, 2020). Regarding GSH depletion, which decreased after one year in the present study, a hypothetical alteration in the pentose phosphate pathway has been pointed out, leading to impaired NADPH production, and resulting in the failure of GSSG to be recycled into GSH by glutathione reductase (GR) (MORENA M, et al., 2002).

Modulation of the NADPH oxidase enzyme complex seems to be a basic requirement to prevent complications in long-term dialysis patients associated with OS (MORENA M, et al., 2002). Moreover, the decrease in GSH can be attributed to a decrease in its synthesis or increased degradation, since it depends on precursor amino acids, such as cysteine, glutamate, and glycine, which may present plasma alterations in HD patients (LU SC, 2013), and that could be due to losses during HD and inadequate protein intake (HENDRIKS FS, et al., 2020). Additionally, GSH is decreased after HD treatment, arising from its increased utilization in order to manage the increased OS during a dialysis session, making patients more susceptible to oxidative damage, and leading to even greater GSH demands (YEH E-L, et al., 2020).

The OS condition may reflect in decreased activity of the erythroid-related nuclear transcription factor 2 (Nrf2) and, consequently, in the expression of antioxidant enzymes such as NAD(P)H dehydrogenase [quinone] 1 (NQO1). Nrf2 is important in the response to OS, as it activates a variety of cytoprotective genes related to the redox status and detoxification, which are impaired in CKD (RAPA SF, et al., 2020).

Elevated intracellular ROS are key contributors to CKD pathogenesis, causing oxidative damage to lipids, DNA (deoxyribonucleic acid), and proteins. In tubular epithelial cells, ROS trigger the release of pro-inflammatory cytokines and chemokines as part of the repair process (IRAZABAL MV and TORRES VE, 2020).

Figure 2 - presents the possible effect of OS in CKD patients undergoing HD after a one-year period.



A: Increased lipid peroxidation assessed by MDA, led to increased GPx enzyme activity. **B:** The increase in GPx enzyme activity led to increased oxidation of GSH to GSSG. **C:** Changes in the pentose phosphate pathway may lead to a decrease in GR enzyme activity. **D:** This decrease in GR may result in a decrease in GSH. **E:** Consequently, there is an accumulation of GSSG. The decrease in GSH can also be attributed to a decrease in its synthesis, which depends on amino acids such as cysteine, glutamate, and glycine, which may present changes in HD patients, besides a decrease associated with the HD process itself, leading to its greater utilization. **F:** The increase in ROS may lead to a decrease in the transcription factor Nrf2, which may decrease the synthesis of antioxidant enzymes such as: SOD, CAT, and NQO1.
Abbreviations: Lipid-OOH: lipid hydroperoxide; Lipid-OH: lipid alcohol; GPx: glutathione peroxidase; GSH: reduced glutathione; GSSG: oxidized glutathione; GR: glutathione reductase; NADPH: reduced nicotinamide adenine dinucleotide phosphate; NADP+: nicotinamide adenine dinucleotide phosphate oxidized; ROS: reactive oxygen species; Nrf2: nuclear factor erythroid 2related factor; SOD: superoxide dismutase; CAT: catalase; NQO1: NAD(P)H dehydrogenase [quinone] 1.
Source: Machado RP, et al., 2026.

As limitations, the current study presents the small size of the convenience sample, which reduces the statistical power of the results and did not allow the sample to be stratified by sex, HD vintage, number of weekly HD sessions (2 or 3 times), comorbidities and age. However, the current study was developed in one dialysis centre and followed-up all patients who were under treatment and not excluded by the exclusion criteria from one year to another. We highlight that the frequency of HD sessions remained unchanged throughout the follow-up, and the same patients were evaluated longitudinally. Furthermore, the absence of glutathione reductase analysis should be considered a limiting factor. The absence of inflammation assessments can be another limitation, considering that OS and systemic inflammation are interconnected processes that can influence and amplify each other.

CONCLUSION

Over the course of one-year, significant alterations in the mechanism of glutathione were observed. Furthermore, the significant increase in MDA levels reinforces the hypothesis that pro-oxidant factors related to CKD, its comorbidities, and the HD procedure itself contribute to the progressive OS observed in this population. While HD plays a crucial role in managing CKD, its long-term impact on oxidative balance underscores the glutathione system as a potential target for therapeutic interventions. Addressing this imbalance remains a key challenge but holds promise for improving patient survival and quality of life. Furthermore, the results of the current study fill a gap in the literature by providing a longitudinal perspective on how chronic HD treatment interacts with oxidative mechanisms, particularly the GSH system. Additional studies with a larger number of patients and extended follow-up periods are needed to better characterize these effects and develop more effective strategies to optimize antioxidant defense in this population.

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