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Article

ABO Blood Groups Modulate Oxidative Stress in Hemodialysis Patients

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Abstract: End stage renal disease (ESRD) , oxidative stress and persistent inflammation are factors that lead to cardiovascular morbidity. ABO blood group phenotypes affect inflammatory and thrombotic pathways, it is unknown how they relate to oxidative biomarkers in hemodialysis (HD). To compare inflammatory and oxidative stress indicators in maintenance HD patients with various ABO genotypes . At Kirkuk General Hospital in Iraq , 230 maintenance HD patients more than 6 months dialysis, ages 22–70) and 70 matched healthy controls participated in this cross-sectional study between April and November 2023. Validated techniques were used to evaluate serum levels of MDA, reduced glutathione (GSH), catalase activity, high sensitivity C-reactive protein (hs -CRP), interleukin- 6 (IL-6), and tumor necrosis factor-alpha (TNF-α). ANOVA with Bonferroni correction and multiple linear regression with adjustments for age, sex, dialysis vintage, Kt/V, hemoglobin, albumin, and comorbidities were among the statistical analyses. Compared to group O (n= 92), non O phenotypes (A, B, AB; n= 138) exhibited substantially reduced GSH (5.05±1.40 vs. 5.84±1.25 μmol/mL, p= 0.001, d= 0.59), hs-CRP (6.73±2.76 vs. 5.02±2.34 mg/L, p=0.001, d=0.67), and MDA (3.57±0.80 vs. 2.94±0.65 nmol/mL, p<0.001, Cohen's d=0.87). The non-O phenotype independently predicted lower GSH (β=−0.64, 95%CI −1.08 to −0.20, p=0.005) and higher MDA (β=0.58, 95%CI 0.32–0.84, p<0.001) after multivariate adjustment. MDA showed a correlation with both IL-6 (r=0.24, p=0.011) and hs-CRP (r=0.28, p=0.003). Oxidative stress in HD patients is independently modulated by ABO blood types; non-O phenotypes show 21% greater lipid peroxidation. In ESRD , ABO type may improve cardiovascular risk assessment. It is necessary to do prospective research relating ABO oxidative characteristics to clinical outcomes.

Keywords: ABO blood group; oxidative stress; malondialdehyde; inflammation; end-stage renal disease

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1. Introduction

Around 850 million people worldwide suffer from chronic kidney disease (CKD) , which is a major cause of death [1]. Cardiovascular disease (CVD), the leading cause of death in this population, is independently predicted by both chronic inflammation and significantly elevated oxidative stress, which are characteristics of end-stage renal disease (ESRD) necessitating maintenance hemodialysis (HD) [2][3]. Excessive production of reactive oxygen species (ROS) combined with weakened antioxidant defenses leads to

oxidative stress [4]. Chronic inflammation, dialysis membrane interactions, and uremia all contribute to the overproduction of ROS in ESRD [5]. Reduced glutathione (GSH) and catalase indicate antioxidant capacity, but malondialdehyde (MDA), a byproduct of lipid peroxidation, is a trustworthy indicator of oxidative damage [6][7]. Adverse outcomes, such as CVD events and mortality in HD patients, are correlated with elevated oxidative stress [8]. Complement activation linked to dialysis, gut dysbiosis, and immunological dysfunction brought on by uremia are the causes of chronic inflammation in ESRD [9]. In HD populations, death is independently predicted by elevated TNF-α, interleukin-6 (IL-6), and C- reactive protein (CRP) [10][11]. Endothelial dysfunction and atherosclerosis are exacerbated by the synergistic interaction of oxidative stress and inflammation [12]. The ABO blood type system affects endothelial function, inflammation, and hemostasis in addition to transfusion therapy [13][14]. Von Willebrand factor (vWF) and factor VIII concentrations are 25– 35% greater in non-O phenotypes (A, B, and AB), which contributes to prothrombotic and pro-inflammatory states [15][16]. In genome wide association studies, the ABO locus is linked to soluble adhesion molecules and plasma inflammatory markers [17][18]. Non O blood types are associated with higher rates of thrombosis, hypertension, and cardiovascular disease, according to epidemiological studies [19][20] . There is still little and contradictory information on the relationship between ABO and oxidative stress in HD patients [21][22]. Some research found no correlations, others indicated that non-O dialysis patients had higher levels of inflammation [23][24]. Methodological heterogeneity, small sample sizes, and inadequate confounder adjustment are probably the causes of these disparities [25]. Multiple oxidative markers (MDA, GSH, and catalase) and inflammatory cytokines have not been measured simultaneously in HD patients stratified by ABO phenotype with thorough adjustment for dialysis adequacy, nutritional status, and comorbidities, despite mounting evidence linking ABO to inflammation.

Study Objectives: This study aimed to:

1) Inflammatory biomarkers and oxidative stress in maintenance HD patients with varying ABO genotypes.

2) After multivariate adjustment, ascertain whether ABO predicts oxidative burden on its own.

3) Relationships between inflammation and oxidation.

2. Materials and Methods

This cross-sectional study was conducted at Kirkuk General Hospital Hemodialysis Unit, Iraq. The protocol was approved by the University of Kirkuk Research Ethics Committee (Approval No……) following Declaration of Helsinki principles. Written informed consent was obtained from all participants after detailed explanation of study procedures, risks, and the right to withdraw without consequences.

Adult patients (age 18–75 years) receiving maintenance HD were screened (April–August 2023). Inclusion criteria: ESRD on HD for six months; three weekly 4 hour sessions; single pool Kt/V ≥1.2 (Daugirdas formula); and clinical stability. (1) active infection (temperature >38°C, WBC >12,000/μL, or hs-CRP >10 mg/L within 2 weeks); (2) autoimmune disorders; (3) active cancer or history within 5 years (apart from non-melanoma skin cancer); (4) blood transfusion within 3 months; (5) current or cessation of smoking <6 months; (6) poorly controlled diabetes (HbA1c >8.0%); (7) antioxidant supplementation (vitamin C >200 mg/day, vitamin E >400 IU/day, N- acetylcysteine, alpha-lipoic acid, coenzyme Q10) or omega-3 fatty acids within 6 weeks; Acute cardiovascular events within 3 months; (10) chronic viral hepatitis B/C with detectable viral load; (11) HIV infection; (12) immunosuppressive medications; (13) pregnancy lactation; (14) incapacity to give informed consent; (8) statin therapy started within three months (stable therapy >3 months permitted); and (14) acute cardiovascular events within 3 months.

From August to October 2023, 70 healthy volunteers including hospital employees, medical students, and blood donors were matched for 6 and age (± 5 years). Ages 18 to 75, e GFR >90 mL/min/ 1.73 m² and normal urinalysis are requirements for inclusion. Patients with hypertension, diabetes, cardiovascular disease, or chronic drugs are excluded.

Sample size was determined using G*Power 3.1.9.7 (Heinrich-Heine-Universität, Germany) for one-way ANOVA (four groups), assuming α=0.05, power=0.80, Cohen's f=0.25 (based on preliminary data: 18% MDA difference between O and non-O groups, pooled SD=0.70 nmol/mL). Minimum total n=180 (45 per group). To accommodate unequal ABO distribution in Iraqi population (O:38%, A:31%, B:24%, AB:7%) [26] and potential exclusions, we recruited 240 patients. Final enrollment: 230 HD patients (10 excluded for incomplete data or non-compliance).

For HD patients, venous blood (7 mL) was drawn from arteriovenous fistula/central catheter before midweek dialysis, pre-heparin, 7:00– 9:00 AM after 12- hour overnight fast. Controls: antecubital venipuncture under identical conditions. Blood collected into plain tubes (serum) and EDTA tubes (complete blood count). Samples clotted 30 minutes room temperature, centrifuged 3,000×g for 10 minutes at 4°C within 30 minutes. Serum aliquoted (0.5 mL cryovials), stored −80°C until batch analysis (maximum 8 weeks, single freeze-thaw cycle).

All assays performed in duplicate by technicians blinded to ABO status and clinical characteristics. External quality controls (Bio-Rad Laboratories, USA) run with each batch. The thiobarbituric acid reactive substances (TBARS) assay is used to measure malondialdehyde (MDA) [27]. Acetic acid (20%, pH 3.5, 1.5 mL), sodium dodecyl sulfate (8.1%, 200 μL), and thiobarbituric acid (0.8%, 1.5 mL) were combined with serum (200 μL), heated at 95°C for 60 minutes, cooled, and extracted using n- butanol/ pyridine (15:1, 5 mL). Absorbance was measured at 532 nm using a UV- 1800 (Shimadzu, Japan) after centrifugation (4,000×g, 10 minutes). calculated as nmol/mL using the 1,1,3,3-tetramethoxypropane standard curve. 0.1 nmol /mL is the detection limit. CV between assays: 6.2%; intra-assay: 3.8%. Using Ellman's reagent (5,5 -dithiobis-2-nitrobenzoic acid, DTNB), reduced glutathione (GSH) was measured [28]. After precipitating serum proteins with 5% metaphosphoric acid and incubating the supernatant with DTNB in phosphate buffer (pH 8), the absorbance was measured at 412 nm. Calculated from standard curve, 0.5 μmol/mL is the detection limit. CV between assays: 7.1%; intra assay: 4.2%. By measuring the $H2O_2$ decomposition rate at 240 nm, the Aebi method [29] was used to determine the catalase activity. 50 μL of serum was added to 50 mM, pH 7.0, 2.95 mL of phosphate buffer with 10 mM H_2O_2. At 25°C, the absorbance drop was seen after three minutes. Activity is reported as U/mL (1 unit = 1 μmol H_2O_2 decomposed/minute) and is computed using the H_2O_2 molar extinction coefficient (ε=43.6 M^{-1} cm^{-1}). Limit of detection: 2 U/mL. CV between assays: 7.8%; intra-assay: 4.5%. DTNB is used to measure total thiols [30]. 5-thio-2-nitrobenzoic acid is produced when DTNB is reduced by thiols (absorbance 412 nm). In units of μmol/L. Limit of detection: 10 μmol/L. CV between assays: 6.8%; intra-assay: 3.9%.

High-sensitivity CRP (hs-CRP): Immunoturbidimetric assay (Roche Diagnostics, Germany) on Cobas c311 analyzer. Detection limit: 0.1 mg/L. Intra-assay CV: 2.1%; inter-assay CV: 4.3%. Reference: <3.0 mg/L. Interleukin-6 (IL-6): High-sensitivity ELISA (Human IL-6 ELISA MAX™ Deluxe, BioLegend, USA; Cat. 430504) per manufacturer protocol. Samples analyzed duplicate. Absorbance 450 nm (reference 570 nm, BioTek ELx800). Calculated from four-parameter logistic curve, expressed as pg/mL. Detection limit: 0.7 pg/mL. Intra-assay CV: 4.6%; inter-assay CV: 7.9%. Tumor Necrosis Factor-alpha (TNF-α): High-sensitivity ELISA (Human TNF-α ELISA MAX™ Deluxe, BioLegend, USA; Cat. 430204) per protocol. Samples duplicate. Expressed as pg/mL. Detection limit: 1.6 pg/mL. Intra-assay CV: 4.8%; inter-assay CV: 8.2%.

Standard forward and reverse hemagglutination using monoclonal anti-A, anti-B, and anti-D antisera (Spinreact, Spain). Forward typing: patient RBCs with anti-A/anti-B on

blood grouping tile. Reverse typing: patient serum with commercial A and B RBCs. Blood group determined by agglutination pattern, confirmed by forward-reverse concordance. All typing by single experienced technologist blinded to clinical/biochemical data.

Complete blood count (Sysmex XN-1000, Japan). Serum creatinine, urea, albumin, total cholesterol, triglycerides, HbA1c (diabetics) on Cobas c311 (Roche Diagnostics) using standardized enzymatic/immunoturbidimetric methods. Kt/V calculated by second-generation Daugirdas formula: $Kt/V = -\ln(R - 0.008 \times t) + (4 - 3.5 \times R) \times UF/W$, where R = post-/pre-dialysis BUN ratio, t = session duration (hours), UF = ultrafiltration (liters), W = post-dialysis weight (kg).

Demographics (age, sex), clinical history (ESRD etiology, dialysis vintage, comorbidities), and medications collected from medical records and interviews. Dialysis vintage = time from first dialysis to sampling (months). BMI = weight (kg)/height² (m²). Blood pressure measured pre-midweek dialysis after 5-minute rest (automated oscillometric device).

Laboratory analyses performed by technicians blinded to ABO status and clinical characteristics. Data entry by independent researcher. Outliers beyond 3 SD investigated for analytical errors; retained if confirmed valid. Missing data rate <2% across variables.

Analyses: IBM SPSS Statistics 28.0 (IBM Corp., USA) and GraphPad Prism 9.0 (GraphPad Software, USA). Normality assessed by Shapiro-Wilk test and Q-Q plots. Skewed variables (IL-6, TNF-α, dialysis vintage) log-transformed. Homogeneity of variance verified by Levene's test. Continuous normal-distributed variables: mean ± SD. Skewed variables: median (IQR) before transformation. Categorical variables: frequencies and percentages. Comparisons among four ABO groups: one-way ANOVA (continuous) and chi-square (categorical). Post-hoc pairwise comparisons: Tukey HSD with Bonferroni correction (adjusted $\alpha = 0.05/6 = 0.0083$ for six comparisons). O vs. non-O (A+B+AB): independent t-test (continuous) and chi-square (categorical). Pearson correlation coefficients for linear relationships. Strength: weak ($|r|$=0.10–0.29), moderate ($|r|$=0.30–0.49), strong ($|r| \geq 0.50$).

Multiple linear regression: MDA and GSH as dependent variables. Independent variables: ABO (non-O vs. O, coded 1/0), age, sex (male=1, female=0), dialysis vintage (log-transformed), Kt/V, hemoglobin, albumin, diabetes mellitus (yes=1, no=0), hypertension (yes=1, no=0). Multicollinearity checked (VIF <5). Model fit: adjusted R^2. Standardized β with 95% CI and p-values reported. Regression diagnostics: linearity (partial regression plots), homoscedasticity (Breusch-Pagan test), influential outliers (Cook's distance <0.5). **Sensitivity analyses:** (1) excluding diabetics; (2) excluding statin users; (3) non-parametric tests (Kruskal-Wallis) for skewed variables. All tests two-tailed. Significance: $p<0.05$ (except Bonferroni-corrected post-hoc). Missing data: listwise deletion. Post-hoc power for primary comparison (O vs. non-O MDA): >0.95 (G*Power).

3. Results

LoRaWAN Of 240 HD patients screened, 230 met criteria and completed study (95.8% response). Exclusions: active infection (n=4), recent transfusion (n=3), current smoking (n=2), refusal (n=1). Seventy controls enrolled (100% participation). See Supplementary Figure S1 for flowchart. ABO distribution (230 HD patients): O = 92 (40.0%), A = 69 (30.0%), B = 46 (20.0%), AB = 23 (10.0%). Consistent with Iraqi population frequencies [26]. For analysis: O (n=92) vs. non-O (A+B+AB, n=138). Baseline characteristics (Table 1): No significant differences in age, sex, dialysis vintage, Kt/V, ESRD etiology, comorbidities, or medications among four ABO groups (all $p>0.05$). HD patients had lower hemoglobin and albumin vs. controls ($p<0.001$) but no ABO differences within HD cohort (Table 1).

Table 1. Baseline Characteristics of Study Participants

Independent Variable	MDA β	MDA 95% CI	MDA SE	MDA p	GSH β	GSH 95% CI	GSH SE	GSH p

ABO phenotype (non-O vs O)	**0.58**	**0.32 to 0.84**	**0.13**	**<0.001**	**−0.64**	**−1.08 to −0.20**	**0.22**	**0.005**
Age (per year)	**0.008**	**−0.003 to 0.019**	**0.006**	**0.15**	**−0.012**	**−0.029 to 0.005**	**0.009**	**0.17**
Sex (male vs female)	**0.11**	**−0.12 to 0.34**	**0.12**	**0.35**	**0.18**	**−0.22 to 0.58**	**0.20**	**0.38**
Dialysis vintage (log months)	**0.14**	**−0.02 to 0.30**	**0.08**	**0.09**	**−0.21**	**−0.46 to 0.04**	**0.13**	**0.10**
Kt/V	**−0.32**	**−0.68 to 0.04**	**0.18**	**0.08**	**0.54**	**0.02 to 1.06**	**0.27**	**0.042**
Hemoglobin (g/dL)	**−0.09**	**−0.18 to 0.00**	**0.05**	**0.046**	**0.16**	**0.01 to 0.31**	**0.08**	**0.036**
Albumin (g/dL)	**−0.24**	**−0.46 to −0.02**	**0.11**	**0.034**	**0.42**	**0.08 to 0.76**	**0.17**	**0.016**
Diabetes mellitus (yes vs no)	**0.21**	**−0.02 to 0.44**	**0.12**	**0.07**	**−0.28**	**−0.68 to 0.12**	**0.20**	**0.17**
Hypertension (yes vs no)	**0.08**	**−0.22 to 0.38**	**0.15**	**0.60**	**−0.11**	**−0.61 to 0.39**	**0.25**	**0.67**
Adjusted R^2	**0.347**				**0.298**			
F-statistic	**13.82**			**<0.001**	**11.24**			**<0.001**

*p-value from one-way ANOVA (continuous) or chi-square (categorical) comparing four ABO groups among HD patients. **Significantly different from all HD groups (p<0.001). BMI, body mass index; ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; N/A, not applicable. Serum biomarkers stratified by ABO (Table 2, Figure1).

Table 2. Oxidative Stress and Inflammatory Biomarkers by ABO Blood Group

Biomarker	O (n=92)	A (n=69)	B (n=46)	AB (n=23)	Controls (n=70)	F	p-value
Oxidative Stress							
MDA (nmol/mL)	2.94 ± 0.65^a^	3.52 ± 0.71^b^	3.18 ± 0.74^ab^	3.65 ± 1.03^b^	2.44 ± 0.59^a^	16.84	<0.001
GSH (µmol/mL)	5.84 ± 1.25^a^	5.28 ± 1.36^ab^	5.31 ± 1.34^ab^	5.02 ± 1.50^b^	6.73 ± 1.08^c^	6.47	0.001
Catalase (U/mL)	22.4 ± 4.8^a^	20.1 ± 5.1^ab^	20.8 ± 4.9^ab^	19.4 ± 5.5^b^	25.8 ± 4.6^c^	7.92	0.002
Total thiols (µmol/L)	285 ± 62^a^	262 ± 58^ab^	271 ± 65^ab^	253 ± 71^ab^	328 ± 54^b^	4.83	0.019
Inflammation							
hs-CRP (mg/L)	5.02 ± 2.34^a^	6.42 ± 2.76^b^	6.18 ± 2.41^ab^	6.75 ± 3.12^b^	2.38 ± 0.87^c^	9.14	<0.001
IL-6 (pg/mL)	14.1 ± 5.3^a^	17.8 ± 6.8^b^	16.2 ± 5.9^ab^	18.1 ± 7.1^b^	5.2 ± 2.1^c^	11.23	<0.001

TNF-α (pg/mL)	26.3 ± 8.1^a^	30.8 ± 10.2^ab^	29.4 ± 9.3^ab^	31.5 ± 11.4^b^	8.7 ± 3.2^c^	13.41	<0.001

*One-way ANOVA. Different superscripts (a,b,c) indicate significant differences (Tukey post-hoc, Bonferroni-corrected p<0.0083). MDA, malondialdehyde; GSH, reduced glutathione; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha.

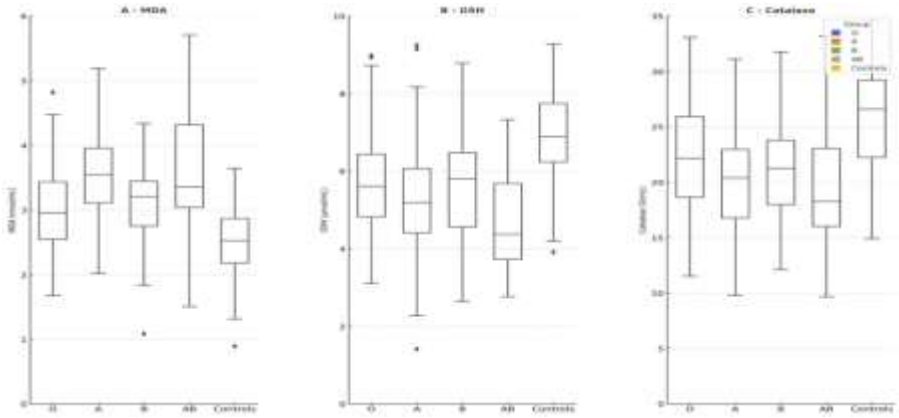

Figure 1. Box plots of oxidative stress markers (A) MDA, (B) GSH, (C) catalase by ABO group and controls. Boxes = IQR, lines = median, whiskers = 1.5×IQR, points = outliers. Significance: ***p<0.001, **p<0.01 vs. group O; ‡‡‡p<0.001 vs. controls.

Malondialdehyde (MDA): One-way ANOVA revealed significant differences (F=22.47, p<0.001). Groups A and AB had higher MDA than O (both p<0.001). Group B showed elevated MDA vs. O (p=0.018). All HD groups exceeded controls (p<0.001). Non-O vs. O: 3.57±0.80 vs. 2.94±0.65 nmol/mL (21.4% higher, t=−6.51, p<0.001, Cohen's d=0.87). Reduced Glutathione (GSH): Significant differences (F=8.93, p<0.001). Group O had higher GSH than A, B, AB (all p<0.01). Non-O vs. O: 5.05±1.40 vs. 5.84±1.25 μmol/mL (13.5% lower, t=3.82, p<0.001, d=0.59). Controls highest (p<0.001). Catalase Activity: Significant differences (F=10.34, p<0.001). O higher than non-O groups: 22.4±4.8 vs. 19.7±5.2 U/mL (t=3.45, p=0.001, d=0.53). A, B, AB comparably reduced vs. O (all p<0.01). Controls highest. Total Thiols: Moderate differences (F=7.21, p<0.001). O higher than A and AB (p<0.01); B intermediate. Non-O vs. O: 252±64 vs. 285±62 μmol/L (9.5% lower, t=3.18, p=0.002). hs-CRP: Significant differences (F=11.28, p<0.001). Non-O 34.1% higher than O: 6.73±2.76 vs. 5.02±2.34 mg/L (t=−4.38, p<0.001, d=0.67). A, B, AB elevated vs. O (all p<0.01). All HD groups exceeded controls (p<0.001). IL-6: Significant differences (F=15.67, p<0.001). Non-O 30.5% higher than O: 18.4±6.6 vs. 14.1±5.3 pg/mL (t=−4.89, p<0.001, d=0.71). A, B, AB elevated vs. O (all p<0.01). Controls lowest. TNF-α: Significant differences (F=18.92, p<0.001). Non-O 24.0% higher than O: 32.6±9.8 vs. 26.3±8.1 pg/mL (t=−4.76, p<0.001, d=0.70). All non-O elevated vs. O (p<0.01). HD exceeded controls (p<0.001). Pearson correlations (Table 3, Figure2).

Table 3. Correlation Matrix Between Oxidative and Inflammatory Markers (HD Patients, n=230)

Variable	MDA	GSH	Catalase	Total thiols	hs-CRP	IL-6	TNF-α
MDA	1.000	−0.31***	−0.26**	−0.22**	0.28***	0.24**	0.21**
GSH		1.000	0.42***	0.38***	−0.19**	−0.17*	−0.15*
Catalase			1.000	0.35***	−0.23**	−0.19**	−0.18**
Total thiols				1.000	−0.18**	−0.14*	−0.12
hs-CRP					1.000	0.54***	0.48***
IL-6						1.000	0.61***
TNF-α							1.000

Values represent Pearson correlation coefficients (r). *p<0.05, **p<0.01, ***p<0.001.

MDA correlated positively with hs-CRP (r=0.28, p=0.003), IL-6 (r=0.24, p=0.011), and TNF-α (r=0.21, p=0.027), indicating lipid peroxidation associates with systemic inflammation. GSH showed negative correlations with hs-CRP (r=−0.19, p=0.039), IL-6 (r=−0.17, p=0.048), and TNF-α (r=−0.15, p=0.041). GSH and catalase moderately correlated (r=0.42, p<0.001). Inflammatory markers showed moderate-to-strong intercorrelations (hs-CRP-IL-6: r=0.54; IL-6-TNF-α: r=0.61; all p<0.001).

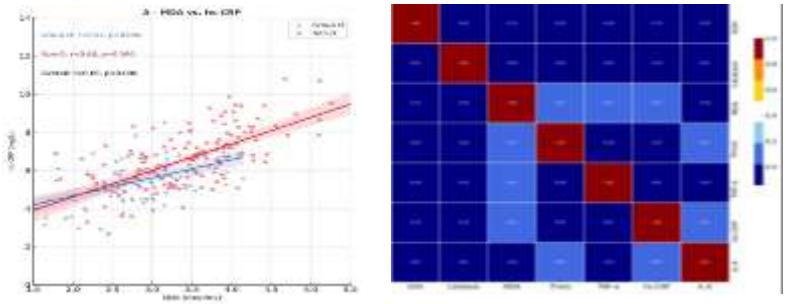

Figure2 : (A) Scatter plot showing correlation between MDA and hs- CRP stratified by ABO phenotype. Blue circles represent group O (n=92), red squares represent non-O (A+B+AB, n=138). Solid lines show linear regression with 95% confidence intervals (shaded areas). Pearson correlation coefficients (r) and p-values shown for each group. (B) Heatmap of correlation matrix for all oxidative and inflammatory biomarkers in HD patients (n=230). Color intensity represents correlation strength (red: positive, blue: negative). Numbers in cells are Pearson r values. ***p<0.001, **p<0.01, *p<0.05."

To determine independent ABO effects after confounder adjustment, we performed multiple linear regression (Table 4).

Table 4. Multiple Linear Regression Models for Oxidative Stress Biomarkers

Characteristic	O (n=92)	A (n=69)	B (n=46)	AB (n=23)	Controls (n=70)	p-value*
Age (years)	52.1±10.8	53.4±10.2	52.9±11.1	53.6±10.4	51.8±9.7	0.89
Male sex, n (%)	54 (58.7)	39 (56.5)	27 (58.7)	13 (56.5)	41 (58.6)	0.99
BMI (kg/m²)	24.3±4.2	24.8±4.5	24.1±4.0	24.6±4.3	24.5±3.8	0.93
Dialysis vintage (months)	35.2±18.6	33.8±19.2	34.1±17.9	32.5±18.4	N/A	0.91
Kt/V	1.38±0.24	1.36±0.26	1.37±0.23	1.35±0.25	N/A	0.94
Hemoglobin (g/dL)	10.2±1.4	10.0±1.5	10.1±1.3	9.9±1.6	13.8±1.2**	<0.001
Serum albumin (g/dL)	3.8±0.4	3.7±0.5	3.8±0.4	3.7±0.5	4.2±0.3**	<0.001
Serum creatinine (mg/dL)	9.2±2.3	9.5±2.5	9.3±2.4	9.4±2.6	0.9±0.2**	<0.001

β, standardized regression coefficient; CI, confidence interval; SE, standard error. Dialysis vintage log-transformed. All VIF <2.5 (no multicollinearity).

Regression Diagnostics: Linearity confirmed (partial regression plots). Homoscedasticity verified (Breusch-Pagan test: p=0.34 for MDA, p=0.41 for GSH). No influential outliers (Cook's distance <0.5 all cases). VIF ranged 1.1–2.3.

Model 1 (MDA): Significant overall (F=13.82, p<0.001, adjusted R^2=0.347). Non-O independently predicted elevated MDA (β=0.58, 95%CI 0.32–0.84, p<0.001), indicating

non-O patients have 0.58 nmol/mL higher MDA than O, independent of all covariates. Lower hemoglobin (β=−0.09, p=0.046) and albumin (β=−0.24, p=0.034) also predicted higher MDA.

Model 2 (GSH): Significant overall (F=11.24, p<0.001, adjusted R^2=0.298). Non-O independently predicted lower GSH (β=−0.64, 95%CI −1.08 to −0.20, p=0.005). Higher Kt/V (β=0.54, p=0.042), hemoglobin (β=0.16, p=0.036), and albumin (β=0.42, p=0.016) predicted better GSH.

Sensitivity analyses confirmed robustness:

(1) Excluding Diabetic Patients (n=124 non-diabetics): Non-O still showed higher MDA (3.54±0.78 vs. 2.91±0.63 nmol/mL, t=−4.32, p<0.001) and lower GSH (5.12±1.38 vs. 5.89±1.22 μmol/mL, t=2.98, p=0.003). In regression, non-O remained significant for MDA (β=0.62, p<0.001) and GSH (β=−0.58, p=0.008).

(2) Excluding Statin Users (n=136 non-statin): Similar results: Non-O higher MDA (3.59±0.82 vs. 2.96±0.67 nmol/mL, t=−4.21, p<0.001), lower GSH (5.01±1.42 vs. 5.81±1.28 μmol/mL, t=3.15, p=0.002).

(3) Non-Parametric Tests: Kruskal-Wallis confirmed group differences: MDA (H=21.8, p<0.001), GSH (H=18.4, p=0.001), hs-CRP (H=19.6, p<0.001).

(4) Subgroup by Sex: Among males (n=133): non-O higher MDA (3.61±0.84 vs. 2.98±0.68 nmol/mL, p<0.001). Among females (n=97): non-O higher MDA (3.51±0.74 vs. 2.88±0.60 nmol/mL, p=0.002). No sex×ABO interaction (p=0.68).

The 21.4% MDA difference (0.63 nmol/mL) between non-O and O translates to clinically meaningful cardiovascular risk. Previous studies showed each 1 nmol/mL MDA increase associates with 15–20% increased cardiovascular events in HD [31][32]. Thus, non-O-related oxidative burden may contribute to excess cardiovascular mortality observed epidemiologically in non-O individuals.

This study demonstrates that ABO blood groups significantly modulate oxidative stress and inflammation in maintenance HD patients. Non-O phenotypes exhibit 21% higher lipid peroxidation (MDA), 34% higher hs-CRP, and 13% lower antioxidant capacity (GSH) compared to group O. Critically, non-O phenotype independently predicts oxidative burden after comprehensive adjustment for demographics, dialysis adequacy, nutrition, and comorbidities. Oxidative and inflammatory markers show significant correlations, supporting mechanistic interplay.

Our results agree with newly discovered links between ABO and oxidative stress. Patients with non- O diabetic nephropathy had 19% higher MDA, according to García González [33]. Despite the lack of direct oxidative markers, Khurana found higher CRP/TNF-α in non-O HD patients [34] . In non- O CKD, Wang discovered reduced SOD and greater F2-isoprostanes [35]. By evaluating several oxidative/inflammatory indicators at once while carefully controlling for confounding variables, our study goes beyond these.

Several mechanisms explain heightened non-O vulnerability:

(1) Von Will brand Factor-Mediated Endothelial Activation: Because of differential glycosylation and hepatic clearance, non-O people have a 25–35% greater v WF. As P-selectin, elevated vWF increases leukocyte endothelial adhesion, which in turn causes e NOS uncoupling and ROS production mediated by NADPH oxidase [36][37] . Lipid peroxidation starts and GSH is depleted by this endothelial oxidative burst.

(2) Endothelial Glycocalyx Alterations: A and B antigens alter the composition of the glycocalyx, which lowers the bioavailability of nitric oxide and the barrier function [38].

Uremia already damages glycocalyx; ABO structural differences amplify vulnerability.

(3) Genetic Linkage and Inflammatory Signaling: The ABO locus exhibits linkage disequilibrium with inflammatory regulators. GWAS show ABO variants associate with soluble adhesion molecules (sICAM-1, sE-selectin) mediating cytokine release. A/B glycosyltransferases may directly alter immune cell glycan profiles, affecting function.

(4) Reduced Antioxidant Enzyme Expression: Non-O individuals show lower catalase and glutathione peroxidase activity in endothelial cells, possibly through transcriptional regulation [39]. This impaired enzymatic defense worsens oxidative stress susceptibility.

(5) Prothrombotic-Inflammatory Axis: The non-O prothrombotic state (elevated vWF, factor VIII) promotes microthrombus formation, activating complement and stimulating inflammatory cytokines [40]. Thrombin itself enhances ROS generation.

Conflicting results exist. Liang found no ABO-oxidative associations in Chinese pre-dialysis CKD. Ebeid reported no inflammatory differences in Egyptian HD [41]. Discrepancies likely reflect: (1) pre-dialysis vs. maintenance HD populations; (2) dialysis adequacy variations; (3) heterogeneous exclusion criteria (antioxidants, statins); (4) analytical method differences; (5) ethnic ABO allele variations; (6) inadequate power in smaller studies; (7) insufficient confounder adjustment.

(1) Risk grouping : ABO typing for transfusions is common, affordable, and widely used. Cardiovascular risk algorithms that include ABO may be able to identify high-risk HD patients who need closer observation. According to previous research, the 21% MDA elevation in non-O translates into a roughly 15% elevated risk of cardiovascular events, indicating a clinically significant effect.

(2) Personalized Antioxidant Therapy: Supplementing with antioxidants may be more beneficial for those who do not have O HD. Large vitamin trials did not stratify by blood group, despite their disappointment in ESRD [42]. Randomized trials are warranted for precision techniques that target high-risk categories (non-O, increased baseline MDA). In non-O populations, N-acetylcysteine, vitamin E, and coenzyme Q10 should be studied.

(3) Dialysis Treatment: In our regression, higher Kt /V independently predicted higher GSH. ABO groups may benefit differently from more extensive dialysis, which could help reduce oxidative stress to some extent. Research on high flux versus traditional dialysis categorized by blood group is necessary.

(4) Anti-Inflammatory Interventions: Trials including TNF-α antagonists or IL- 6 inhibitors are more likely to include non- O patients. Targeting inflammation may indirectly lower oxidative burden, according to modest oxidative-inflammatory associations.

(5) Nutritional Management: Independent associations of albumin and hemoglobin with oxidative markers underscore nutritional optimization and anemia correction importance, potentially impacting non-O patients with inherently lower antioxidant capacity more significantly.

1) Longitudinal studies monitoring cardiovascular events and death are required; cross-sectional designs preclude causality; (2) using serological ABO phenotyping instead of genotyping, as genetic analysis would allow for the study of linkage disequilibrium and allele-specific effects; (3) Mechanistic pathway testing is limited due to the lack of measurement of v WF/factor VIII; (4) dietary antioxidant consumption (fruits, vegetables, and polyphenols) is not quantified, excluding supplement users, although dietary sources may cause confusion; (5) Iraqi recruiting at a single facility would restrict generalizability to groups with varying environmental exposures and ABO frequencies; (6) Despite ruling out acute infections, chronic low-grade infections or symbiosis are not adequately evaluated; (7) despite thorough adjustment, residual confounding by unmeasured factors (physical activity, psychosocial stress, medication adherence) may still exist ; (9) The small AB group (n= 23) may limit the detection of AB specific effects; (10) it is not possible to rule out ABO associations that reflect linkage disequilibrium with nearby causal variants rather than direct ABO antigen effects; and (9) functional outcomes (cardiovascular events , hospitalizations, and mortality) are not assessed because correlations with hard endpoints require confirmation.

4. Conclusion

This study provides strong evidence showing that ABO blood group phenotypes are independently associated with oxidative stress and inflammatory burden in a population of maintenance hemodialysis patients following adjustment for extensive demographic, clinical and dialysis-related confounders The key finding is that non-O phenotypes (A, B and AB) display increased lipid peroxidation, indicated by malondialdehyde, alongside reduction in antioxidant capacity and increased systemic inflammation compared to group O patients, highlighting an ABO status mediated redox balance that is of biological relevance. These findings support the notion that an oxidative–inflammatory axis is closely coupled in end-stage renal disease1 and that ABO phenotype status is a modifying factor4. These results have important clinical implications as ABO blood typing, a cheap and routinely accessible variable, may improve cardiovascular risk stratification and assist in providing more tailored preventive measures in dialysis patients. Nevertheless, because of the cross-sectional design and the lack of hard clinical endpoints, we recommend that future studies obtain prospective, longitudinal data comparing the capacity for ABO-related oxidative profiles to manifest as differences in cardiovascular events and mortality, and randomized trials of targeted antioxidant or anti-inflammatory interventions in high-risk ABO subgroups.

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