Cardiovascular risk of circulating endotoxin level in prevalent hemodialysis patients

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Abstract

Background: Cardiovascular diseases (CVDs) are a major cause of morbidity and mortality in patients with end stage renal disease (ESRD). Circulating endotoxins may have toxic effect on myocardial functions and are speculated as pathogens of accelerated atherosclerosis and hemodialysis (HD) patients. Objective: We aimed to assess the possible relation between circulating endotoxin levels and left ventricular functions parameters, common carotid artery intimal media thickness (CIMT) in prevalent HD patients.

Patients and Methods: Forty stable prevalent HD patients with mean age (47.97 ± 14.42) year using regular conventional hemodialysis sessions in Ain shams university hemodialysis unit, Cairo, Egypt were randomly selected. Diabetics, congestive heart failure and those with history of myocardial infarction or coronary artery disease were excluded from the study. All patients were studied by CBC and routine chemistry, as well as hs CRP, Intact PTH, lipid profile and endotoxin level by ELISA before and after the HD session, Delta change of endotoxin (pre dialysis endotoxin-post dialysis endotoxin) was calculated, resting Doppler echocardiographic and carotid duplex.

Results: Mean of Pre-HD session serum endotoxin level was (0.356 ± 0.090) EU/mL and the mean of post-HD endotoxin levels was (0.367 ± 0.110) EU/mL. Significant positive correlation between post dialysis endotoxin, MV E/A ratio and grades of left ventricular diastolic dysfunction (P < 0.05) and significant correlation between delta change in endotoxin and EF% (r = −0.36, P = 0.02). By stepwise linear regression analysis for determinants of MVE/A post-HD endotoxin level independently associated with MV E/A ratio (β = 0.350, P = 0.027). We did not detect any significant correlation between CCA atherosclerosis and neither pre nor post-HD endotoxin level nor with delta change of pre and post HD endotoxin levels.

Conclusion: Acute increase in post dialytic circulating endotoxin level in prevalent HD patients may be associated with both left ventricular systolic and diastolic dysfunction and that attempts to reduce endotoxin level may have a positive impact on cardiovascular complications in HD Patients.

1. Introduction

Cardiovascular diseases (CVDs) are a major cause of morbidity and mortality in patients with end stage renal disease (ESRD). CVDs are the cause of death in hemodialysis (HD) patients accounting for 40%–45% of all deaths.1 Endotoxemia results in a broad range of negative CV effects and may explain increased cardiovascular deaths rate in HD patients.2 Endotoxin is typically used to describe a complex of protein and lipopolysaccharides (LPS) molecules found in the outer cell wall of gram-negative bacteria that either slough off during growth, or released upon cell lysis.3 The presence of endotoxin in HD patients may be attributed to dialysate contamination, but many studies support its endogenous origin from the gut as they reported the presence of gut bacterial DNA fragments in the blood of chronic kidney disease (CKD) patients maintained on HD and in CKD patients who did not receive dialysis treatment.4 The presence of endotoxemia points to impairment of intestinal barrier structure and function in ESRD patients as influx of urea into intestinal tract may be attributed to the pathogenesis of intestinal barrier dysfunction.5 The precise mechanism by which HD aggravates endotoxemia in ESRD patients remains unknown.6 Gut edema,7 alteration in
hemodynamics during HD induces intestinal mucosa ischemia, which aggravates intestinal mucosa impairment and vascular access as tunneled catheter with bacterial biofilm resulting in bacteremia and endotoxia in addition to dialysate contamination are factors that may aggravate endotoxia in HD. Exposure to high levels of bacteria and endotoxin is clearly associated with short-term complications, ranging from pyrogenic reactions to septicemia. In addition, long-term endotoxin challenge may also promote a state of chronic inflammation with subsequent frequent complications including increased risk of CVDs, hospitalization, and death in dialysis patients. We aimed to assess possible relation between resting Doppler echocardiographic left ventricular parameters carotid duplex parameters of atherosclerosis and circulating endotoxin levels in prevalent HD patients.

2. Patients and methods

This was across sectional study that included randomly selected forty hemodialysis (HD) patients recruited from Ain Shams University hospital, hemodialysis unit, Cairo, Egypt. This research has been approved by the ethical committee in Ain Shams University Hospital. Patients were clinically stable, on regular conventional hemodialysis sessions thrice weekly >6 m duration. Each dialysis session lasted four hours using bicarbonate dialysate, low flux polysulfone dialyzer and heparin as anticoagulant. The following subjects were excluded from the study: patients with diabetes mellitus, congestive heart failure, those with evident history of coronary heart disease, uncontrolled hypertension, malignancy, and advanced liver disease or acute infection. Baseline demographic data were collected [Age, sex, Body mass index (BMI) (kg/m²), etiology of renal failure, duration of HD, vascular access, Dry weight (kg), ultrafiltration (UF) volume (L) on the session, UF rate (UFR) (ml/h/kg body weight), the average of Pre-HD systolic and diastolic BP (mmHg) per week and mean arterial BP (MAP; mmHg)] were calculated.

2.1. Laboratory investigations

Biochemical blood samples were collected before the midweek HD session and before heparin administration with the exception of the post-dialysis urea level and post-dialysis serum endotoxin level. Laboratory tests done for all patients included [hemoglobin (Hgb), hematocrit, serum iron profile, creatinine, sodium (Na), potassium (K), blood urea nitrogen (BUN), calcium (Ca), phosphate (P), intact PTH (iPTH), Albumin, lipid profile (Total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) and triglycerides (TG)) and high-sensitivity CRP (hsCRP)]. Urea reduction ratio (URR) was calculated using pre-dialysis urea (U pre) and post-dialysis urea (U post). URR = Upre - Upost / Upost X 100/Upre.

Endotoxin serum levels were measured in post-dialysis and post-dialysis samples. In addition, delta change of endotoxin level was calculated as (pre-dialysis endotoxin – post-dialysis endotoxin) serum endotoxin level quantification; Endotoxin Units per milliliter (EU/mL) was assayed using Human Endotoxin ELISA kit, Glory Science Co., Ltd, USA. Detection range is 0.02 EU/mL–0.8 EU/mL. Coagulated serum samples were kept at room temperature for 10–20 min, centrifuged at the speed of 2000–3000 rpm for 20 min and supernatant was removed. A standard curve was plotted on a graph paper (standard concentration as the horizontal and optical density value for the vertical). The sample concentration was detected according to sample optical density. Detection of endotoxin in the dialysate was done using Endotoxin Testing Kit for Dialysis Water and Dialysate, Xiamen Bioendo Technology Co., Ltd, China (Gel Clot Assay; Catalog Number: GD010060; sensitivity 0.125 EU/ml).

2.2. Transthoracic 2D echocardiography

Echocardiography study was done by a single experienced cardiologist during the midweek dialysis day. The assessment of LV geometry was obtained by 2D image, with the following variables: LV end-diastolic diameter (LVEDD), LV end-systolic diameter (LVESD), LV posterior wall diameter in diastole (LVPWd), Interventricular septum diameter in diastole (IVSd).

The LV mass (LVM; g) was calculated using the formula: LVM = 0.8[(LVEDD + IVSd + PWD/3 - LVESD3)] + 0.6. LVM was indexed to body surface area (BSA), to obtain the LV mass index (LVMI). (LVMI = LVM/BSA; g/m²).

LVH was diagnosed when LVMI was >115 g/m² for men and >95 g/m² for women.

In addition, LV relative wall thickness (RWT) was calculated using the equation:

\[\text{RWT} = \frac{2 \times \text{PWT}}{\text{LVEDD}}\]

Mitrail flow was measured in apical four-chamber view by pulsed Doppler. The following variables were obtained: early (E) and late (A) trans-mitral diastolic velocities and E/A ratio.

Tissue Doppler was performed in the apical four-chamber view to obtain the velocities of the mitral annulus. The sample was placed at the junction of the LV lateral wall with the mitral annulus, and then early (e’) diastolic velocities of the mitral annulus were identified, as well as the E/e’ ratio.

LV Diastolic function was assessed using several parameters including the pattern of mitral inflow and the ratio of peak early (E) filling velocity to late diastolic filling (A) velocity (E/A ratio), deceleration time of early filling velocity (DT), and the isovolumic relaxation time (IVRT). PW Tissue Doppler Imaging was performed in the apical views to acquire mitral annular velocities. The ratio of early mitral inflow velocity to Tissue Doppler velocity e’ (E/e’) was used for the estimation of LV filling pressures. Diastolic function was classified into grades I–IV: normal, grade I (abnormal relaxation, grade II (pseudo-normal pattern) and grade III (restrictive pattern). It was considered grade I when E/A < 1; grade II when E/A > 2 and grade II when E/A was >1 and <2 in association with E/e’ > 10.

2.3. Carotid Doppler study

We used Carotid artery Doppler Albion Korea E cube 9 machine using both B-mode and Doppler settings. All patients were examined in supine position using linear transducer (8–12 MHz) with the neck extended and head tilted toward the opposite of the examined site. Using B-mode, the arterial wall composed of two parallel echogenic lines separated by a hypoechoic space. The carotid intima-media thickness (CIMT) represents the combined thickness of the hypoechoic space plus the hyperechoic lines. The CIMT was calculated on each side at the distal part of the CCA 1–2 cm proximal to the level of the bulb and usually we used the far wall for measurement (farthest wall to the transducer) to obtain more accurate results. Generally, values of CIMT ≥ 0.9 mm were considered abnormal. Mean CIMT (CIMT mean) values form both the right and the left CCA were calculated. Also, the CIMT max was also calculated. Also, using the B-mode we assessed each patient for the presence or absence of plaques and reporting their nature, size, location and effect.

Doppler study was done for all patients for assessment of any suspicious stenosis. The degree of diameter stenosis and its location were reported. Generally, diameter stenosis >65–70% is considered significant (according to the most major vascular centers and this is the level need surgical interference).

Both echocardiography assessment and carotid duplex were done at the same day of withdrawing samples of serum endotoxin post-dialysis session.
2.4. Statistical methods

Analysis of data was done by IBM computer using SPSS (Statistical Program for Social Science) version 18. Quantitative data were presented as minimum, maximum, mean and SD. Qualitative data were presented as number and percent. Chi-square test was used to compare qualitative variables between groups. One way ANOVA (analysis of variance) test was used to compare quantitative variables between more than two groups. Logistic regression analysis was used for determinants of MVE/A. P value less than 0.05 was considered statistically significant.

3. Results

The study included 40 prevalent hemodialysis patients, 13 (32.5%) out of them were females and 27 (67.5%) were males with mean age of 47.97 ± 14.42 year range (20–70). Mean duration of hemodialysis (HD) was 78.15 ± 65.30 months with average 3 sessions per week the majority of patients 37(92.5%) with arteriovenous fistula (AVF) as vascular access and mean amount of heparin used in IU (as anticoagulant through HD sessions) was 5062.50 ± 856.26 IU. The etiology of ESRD was hypertension in 16 (40%) patients, 6 (15%) Chronic glomerulonephritis (GN) in 6 (15%) patients, 3 (7.5%) Obstructive uropathy in 3 (7.5%) patients, 4 (10%) polycystic kidney disease (PKD) in 4 (10%) patients, and amyloidosis in one (2.5%) patient. 26 (65%) of patients were hepatitis C virus (HCV) positive serology. The demographic and laboratory data of the studied patients are shown in Tables 1 and 2. We didn’t observe any change in pre-dialysis endotoxin level.

The results revealed that endotoxin level did not change in 16 (40%) patients, decreased in 12 (30%) and increased in 12 (30%) patients. The demographic and laboratory data of the studied groups are shown in Tables 1 and 2. The results revealed that endotoxin level did not change in 16 (40%) patients, decreased in 12 (30%) and increased in 12 (30%) patients. The demographic and laboratory data of the studied patients are shown in Tables 1 and 2. We didn’t observe any change in pre-dialysis endotoxin level.

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increased endotoxin post HD session regarding sex, etiology of renal failure, vascular access, BMI, ultrafiltration volume, hemodialysis duration or HCV status or studied laboratories (P > 0.05).

Pre-dialysis endotoxin level was significantly negatively correlated to S. Albumin (r = −0.38, P = 0.01). Age was significantly correlated to post-dialysis endotoxin level and with delta change of endotoxin (r = 0.35, P = 0.026). Correlations of pre-dialysis and post-dialysis endotoxins levels with studied laboratories were shown in Table 2.

### Table 3

| Post-dialysis endotoxin (EU/mL) | 0.3675 | 0.11010 | Pearson correlation | P value |
|---------------------------------|--------|---------|---------------------|---------|
| EF %                            | 61.0000| 7.48674 | 0.01                | 0.96    |
| IVSd cm                         | 1.0750 | 0.26675 | −0.08               | 0.64    |
| LVPPWd cm                       | 1.0650 | 0.26462 | −0.26               | 0.11    |
| LVEDD cm                        | 5.1175 | 0.89611 | 0.02                | 0.88    |
| LVESD cm                        | 3.4725 | 0.68550 | 0.003               | 0.99    |
| LV mass gm                      | 220.2500| 85.26843| −0.16               | 0.34    |
| LV mass index g/m²              | 125.2500| 45.38143| −0.08               | 0.62    |
| RWT                             | 0.4185 | 0.11648 | −0.20               | 0.23    |
| Peak Pa AV                      | 11.0655| 11.08032| −0.10               | 0.55    |
| MV E vel m/s                    | 0.8227 | 0.19877 | 0.14                | 0.40    |
| MV A vel m/s                    | 0.8038 | 0.20432 | −0.20               | 0.23    |
| MV E/A ratio                    | 1.0690 | 0.37700 | 0.35                | 0.03    |
| e’ m/s                          | 0.1077 | 0.02547 | 0.18                | 0.26    |
| E/e’ ratio                      | 8.0553 | 2.66837 | −0.02               | 0.92    |
| Post–dialysis endotoxin         |        |         |                     |         |
| Diastolic dysfunction           |        |         |                     |         |
| Mean                            | 0.3591 | 0.0917  | 3.18                | 0.04    |
| SD                              | 0.3458 | 0.0920  |                     |         |
| grade I                         | 0.4500 | 0.0866  |                     |         |
| grade II                        | 0.3500 | 0.2828  |                     |         |
| grade III                       |        |         |                     |         |

* Using Post Hoc test (Tukey) test, the significance is between grade III and both normal and grade I groups.

**EF**: ejection fraction, **IVSd**: interventricular septum diameter (diastole), **LVPWd**: left ventricular posterior wall diameter (diastole), **LVEDD**: left ventricular end-diastolic diameter, **LVESD**: left ventricular end-systolic diameter, **RWT**: relative wall thickness.

### 3.1. Circulating endotoxins and LV functions by echocardiography

There was significant correlation between post-endotoxin level and E/A ratio (r = 0.35, P < 0.05) and positively significant with the grade of left ventricular diastolic dysfunction (P < 0.05) (Table 3) (Fig. 1). By stepwise linear regression analysis for determinants of MVE/A, post HD endotoxin level was found to be independently associated with MV E/A ratio (B = 0.350, P = 0.027). Delta endotoxin level was significantly negatively correlated to EF (r = −0.36, P = 0.02) (Fig. 2), LVEDS cm (r = 0.36, P = 0.02) and MV A vel m/s (r = −0.38, P = 0.02) (Table 4).
**Fig. 2.** Correlation between endotoxin delta change and ejection fraction.

### Table 4
Correlation between endotoxin delta change and echo parameters.

|                          | Mean    | SD      | Pearson correlation | *P* value |
|--------------------------|---------|---------|--------------------|-----------|
| Endotoxin delta change   | 0.0837  | 0.09499 |                    |           |
| EF %                     | 61.0000 | 7.48674 | −0.36              | 0.02      |
| IVSd cm                  | 1.0750  | 0.26675 | 0.04               | 0.81      |
| LVPWd cm                 | 1.0650  | 0.26462 | 0.06               | 0.70      |
| LVEDD cm                 | 3.1175  | 0.89611 | 0.24               | 0.14      |
| LVESD cm                 | 3.4725  | 0.68650 | 0.36               | 0.02      |
| LV mass gm               | 220.2500| 85.26843| 0.13               | 0.43      |
| LV mass index g/m²       | 125.2500| 45.38143| 0.13               | 0.41      |
| RWT                      | 0.4185  | 0.11648 | −0.08              | 0.64      |
| Peak Pa AV               | 11.0655 | 11.08032| −0.15              | 0.34      |
| MV E vel m/s             | 0.8227  | 0.19877 | −0.24              | 0.14      |
| MV A vel m/s             | 0.8038  | 0.20432 | −0.38              | 0.02      |
| MV E/A ratio             | 1.0690  | 0.37700 | 0.17               | 0.29      |
| e' m/s                   | 0.1077  | 0.02547 | −0.01              | 0.98      |
| E/e' ratio               | 8.0553  | 2.66837 | −0.17              | 0.29      |

EF: ejection fraction, IVSd: interventricular septum diameter (diastole), LVPWd: left ventricular posterior wall diameter (diastole), LVEDD: left ventricular end-diastolic diameter, LVESD: left ventricular end-systolic diameter, RWT: relative wall thickness.

* Statistically significant values.

### Table 5
Correlation between CCA Atherosclerosis and circulating Endotoxin levels.

| CCA atherosclerosis | T test | *P* value |
|---------------------|--------|-----------|
|                     | Mean   | SD        | Mean   | SD   |
| No (n = 25)         |        |           |        |      |
| Endotoxin pre       | 0.3635 | 0.08895   | 0.3429 | 0.09376 | 0.019 | 0.891 |
| Endotoxin post      | 0.3577 | 0.08330   | 0.3857 | 0.14991 | 0.700 | 0.48  |
| Yes (n = 15)        |        |           |        |      |

Correlation between CCA intimal thickness

|                          | Mean | SD |
|--------------------------|------|----|
| Endotoxin pre            | 0.3562 |   |
| Endotoxin post           | 0.3675 |   |
| Endotoxin delta change   | 0.0112 |   |

*Statistically significant values.*
3.2. Circulating endotoxins and CCA atherosclerosis

The mean of CIMT was 0.940 ± 0.3037 mm among studied HD patients; CCA Atherosclerotic changes CIMT > 0.9 mm in 15 (35.0%) of the total patients (8 patients with atheromatous plaques, 2 patients with non-significant Carotid stenosis and one patient with significant stenosis) and 25 (65.0%) were within normal. There was no significant correlation between CCA atherosclerosis parameters and either pre or post- HD endotoxin level nor with delta change of pre and post HD endotoxin levels (Table 5).

4. Discussion

This study showed that acute increase in post dialytic circulating endotoxin level in HD patients is associated with both left ventricular systolic and diastolic dysfunction and can hence participate in higher cardiovascular events in HD patients. Circulating endotoxins was reported to be increased in patients with advanced (CKD) stages especially those on regular hemodialysis, and may be considered a pathogen of cardiovascular complications in those patients. LV diastolic dysfunction is proven to be a significant predictor of mortality in patients with CKD.

In the present study, we reported a correlation between low-grade endotoxia induced by HD, as demonstrated by post-dialysis endotoxin level, and impaired LV diastolic function in HD population. There was significant positive correlation between post-dialysis endotoxin level both early/late trans-mitral diastolic velocities (MV E/A ratio) and LV diastolic dysfunction grades. Delta change of endotoxin level throughout HD session (pre endotoxin-post HD endotoxin) was significantly negatively correlated to late diastolic filling velocity MV A vel (m/s); a parameter that usually decreases with advanced diastolic dysfunction ($P < 0.05$).

A possible explanation for the correlation between post-dialysis endotoxin level and LV diastolic function can be explained through the effect of UF and hemodynamic stress during HD therapy that results in decreased mesenteric blood flow with subsequent increase in gut mucosal permeability and endotoxin translocation. Some theories explain the occurrence of myocardial dysfunction after endotoxin action that seems to binding of lipopolysaccharide (LPS) endotoxin to Toll-like receptor (TLR) type 4 and CD-14 on cardiomyocytes that evoke rapid innate immune response and release of cytokines and myocardium dysfunction during endotoxia.

There was a significant increase in delta change of endotoxin level through the hemodialysis session associated with decreased in EPI. The LVEF is affected by LV contractility, preload and afterload as exposure to endotoxin, as proinflammatory stimulus, may provoke a release of a wide variety of proinflammatory cytokines leading to peripheral vasodilation and reduction in contractile performance of myocardium.

On the other hand, McIntyre et al.'s study reported that pre-dialysis endotoxin level showed a statistically significant correlation with myocardial stunning severity and pre-dialysis serum cardiac troponin T (cTnT). Brown et al.'s study also showed LV diastolic and decreased MV E/A ratio associated with endotoxia but with severe sepsis.

Endotoxia results in synergistic up-regulation of proinflammatory genes in the macrophages that may enhance the process of inflammatory activation within the atherosclerotic lesions. However, we didn't detect any statistically significant correlation between CIMT, the presence of atheromatous plaque or carotid significant stenosis and endotoxin level pre or post HD session in our studied population. This might be attributed to the relatively small studied patients' sample. Szeto, on the other hand, reported a significant correlation between serum endotoxin level and both carotid intimal thickness and atherosclerotic changes in peritoneal dialysis patients.

The present study explored a statistically significant negative correlation between pre-dialysis endotoxin level and serum albumin level ($P = 0.01$). Albumin has anti-inflammatory properties by binding to endotoxin and reducing the expression of proinflammation markers, and a similar correlation was reported in CKD and peritoneal dialysis patients.

We found significant positive correlation between age and delta change of endotoxin level in HD session in comparison with Feroze et al.'s study who reported that older HD patients had a higher level of serum endotoxin. However, they didn't report significant correlation between serum endotoxin level and the age. The Lipopolysaccharide-binding protein was increased with age, and the mechanisms underlying such an increase are not known. Intestinal permeability and subsequent bacterial translocation increase with age in experimental models.

Endotoxin level in ESRD patients on regular hemodialysis may contribute to many factors as gut hypoperfusion and bowel edema, which induces permeability changes and allows bacterial translocation across the intestinal lumen to blood stream and bacterioly-sis induced by systemic antibiotics, potential use of non-ultra-pure dialysate (Endotoxin level threshold of <0.03 EU/mL) for the dialysis, circulatory oxidative stress. Also many gut mucosal dysfunctions as malabsorption, loss of permeability control, food constituents, food additives and gut inflammation may also contribute to endogenous gut endotoxin formation.

We observed the change in the serum endotoxin after HD session, as 30% of patients showed increased endotoxin and others showed decreased or no change in contrast to McIntyre et al.’s study who didn’t observe change in endotoxin level during hemodialysis session. This may be contributed to many factors affecting endotoxin level from patients to another, and also as post-dialysis period is the time of endotoxin translocation predominantly. Despite of the variation of delta change in endotoxin level we observed in the study that post-endotoxin level and delta change of endotoxin among HD session were with negative impact on diastolic and systolic function of the left ventricle in prevalent HD patients.

5. Conclusion

Acute increase in post dialytic circulating endotoxin level in prevalent HD patients may be associated with both left ventricular systolic and diastolic dysfunction, and applying strategies to reduce endotoxin level may have a positive impact on cardiovascular complications in HD Patients.

Conflict of interest

We have no conflict of interest to declare.

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