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Original Article

Cardiovascular risk of circulating endotoxin level in prevalent hemodialysis patients

Mohamed Ibrahim [a], Maha Behairy [a], Marwa El-Ashry [b], Ahmad E. Mostafa [c,*]

[a] Internal Medicine and Nephrology Department – Ain Shams University, Egypt
[b] Clinical and Chemical Pathology Department – Ain Shams University, Egypt
[c] Cardiology department, Ain Shams University, Egypt

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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 (ß = 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

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* Corresponding author.
E-mail address: ahmad_sayedyousef@yahoo.com (A.E. Mostafa).

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hemodynamics during HD induces intestinal mucosa ischemia, which aggravates intestinal mucosa impairment[8] and vascular access as tunneled catheter with bacterial biofilm resulting in bacteremia and endotoxemia[9] in addition to dialysate contamination[10] are factors that may aggravate endotoxemia 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[11] including increased risk of CVDs, hospitalization, and death in dialysis patients.[12] 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 weakly >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/m^2), 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 X 100/Upre.

Endotoxin serum levels were measured in pre-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 plot 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{1.04[([LVEDD + IVSd + PWd]3 - LVEDD3)]} + 0.6. LVM was indexed to body surface area (BSA), to obtain the LV mass index (LVMI). (LVMI = LVM/BSA; g/m^2).[13]

LVH was diagnosed when LVMI was >115 g/m^2 for men and >95 g/m^2 for women.

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

RWT = 2 * PWd/LVEDD. Mitral 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 filing (*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 III when *E*/*A* > 2 and grade II when *E*/*A* was >1 and <2 in association with *E*/*e*' > 10.[14]

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 $\geq$ 0.9 mm were considered abnormal.[15] 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 echocardiograph assessment and carotid duplex were done at the same day of withdrawing samples of serum endotoxin post-dialysis session.

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