Intradialytic Hypotension: Beyond Hemodynamics

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Summary

Intradialytic Hypotension is a major complication during hemodialysis session, associated with increased risk of cardiovascular events and mortality. Its pathophysiology is believed to be multifactorial and remains not well elucidated. The aim of this study is to put forward new mechanisms behind the development of intradialytic hypotension. The study included sixty-five subjects on chronic hemodialysis, divided into two groups: intradialytic hypotensive (n=12) and normotensive (n=53), according to the variation of systolic blood pressure between post-dialysis and pre-dialysis measurements. Renin and angiotensin converting enzyme I plasma concentrations increased in both groups but more likely in normotensive group. Aldosterone plasma concentration is increased in the normotensive group while it decreased in the intradialytic hypotension group. Plasma endothelin concentrations showed higher values in intradialytic hypotension group. Post-dialysis asymmetric dimethylarginine and angiotensin converting enzyme 2 plasma concentrations were significantly higher in intradialytic hypotension group as compared to normotensive one. Collectrin plasma concentrations were significantly lower in intradialytic hypotension group. Finally, post-dialysis vascular endothelial growth factor C plasma concentration significantly increased in intradialytic hypotension group. In conclusion, endothelial dysfunction characterized by a lower level of vasoactive molecule seems to play a critical role in intradialytic hypotension development.

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Introduction

Intradialytic Hypotension (IHO) is the most common complication occurring in 5 and 30 % of hemodialysis (HD) sessions (Chou *et al.* 2017). The National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines and the 2007 European best practices guidelines (Kooman *et al.* 2007), define IHO as a decrease in either systolic blood pressure (SBP) by more than 20 mm Hg or mean arterial pressure by more than 10 mm Hg, associated with symptoms of hypoperfusion including nausea, vomiting, abdominal discomfort yawning, sighing, muscle crumps, restlessness, dizziness, fainting and anxiety (K/DOQI Workgroup 2005, Gul *et al.* 2016). IHO is known to lead impairment of patient's quality of life (Ronco *et al.* 2000). In addition, frequent episodes of IHO may result in cardiovascular complications e.g. ischemic heart events, cardiac arrhythmia, and congestive heart failure

(McIntyre 2010), leading to increased hospitalization, and increase in mortality rate (Inrig *et al*. 2007, Chou *et al*. 2017, Chou *et al*. 2018). Despite the different strategies in treating and preventing IHO (Reeves and Mc Causland 2018) (real-time hemodynamic monitoring, volumetric control, bicarbonate-based buffer, and biocompatible membranes), it remains a common and distressful event during HD procedure. In general, the pathophysiology of IHO is etiology dependent. Most of the studies suggest acute hypovolemia induced by rapid removal of blood volume as a major factor, along with an inadequate response of the cardiovascular system in reestablishing a normal blood volume (Daugirdas 2001, Palmer and Henrich 2008, Chou *et al*. 2017). Nevertheless, the pathophysiology of IHO is believed to be multifactorial. Therefore, therapeutic interventions to correct IHO should be more adjusted, taking into consideration all the pathophysiological mechanisms. The Renin Angiotensin Aldosterone system (RAAS), nitric oxide (NO) and other endothelial molecules are implicated in blood pressure control (Levine *et al*. 2012). Their role in the pathophysiology of IHO is not yet elucidated. Hence, the better understanding of the pathophysiological mechanisms underlying IHO could be of a great interest. The objective of our study is to assess the endothelial microenvironment molecules status in IHO patients in order to better understand the pattern of this entity and to pinpoint new therapeutic strategies.

Methods

Study design outcome

The protocol was approved by the Ethical Committee of the Saint Joseph University and the Hotel Dieu de France University Hospital (Beirut) according to the declaration of Helsinki. All HD patients within the dialysis unit of the tertiary university hospital (Hotel Dieu de France, Beirut) were invited to take part of this study. The criteria to be eligible for the study were patients on chronic HD, >18 years of age, medically stable, undergoing hemodialysis three times per week for a minimum of 4 h per session and for more than 6 months. Patients with active infection, pulmonary diseases, heart failure (left ventricular ejection fraction <50 %), cancer, or any other terminal illness were excluded from the study. 80 patients were initially eligible for the study, 65 subjects were enrolled following inclusion/exclusion criteria (Fig. 1).

Dialytic session data were recorded for three months period study, on a session-to-session basis. Laboratory parameters were measured monthly. Body temperature and heart rate were taken before and after each HD session. Excess weight at the start of dialysis was defined as the difference between pre-dialysis weight and dry weight. The prescribed ultrafiltration (UF) volume was calculated by adding the estimated intradialytic fluid intake (usually 500-750 ml) to the excess weight. However, for all analyses, the exact UF volume as delivered by the dialysis apparatus was used. Blood pressure was monitored using an automated sphygmomanometer integrated in the dialysis B Braun machine, before, hourly during and immediately following the dialysis session or when hypotensive symptoms occur. Pre-dialysis blood pressure was measured in the non-access arm at the beginning of HD after 10-minutes rest with the patient seated in the dialysis chair before placement of a dialysis needle. Post-dialysis blood pressure was measured at the end of the session, 10-minutes after disconnecting the patient from the dialysis circuit.
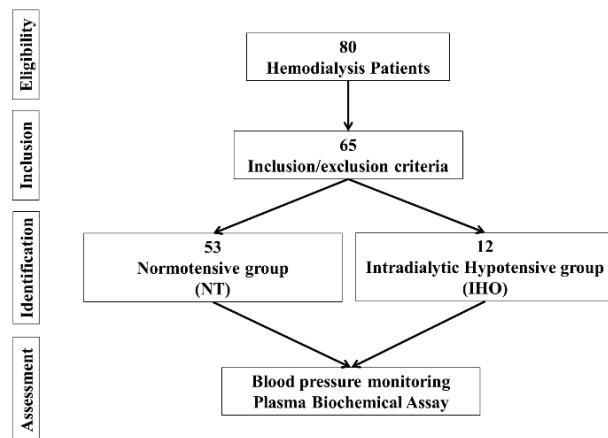

Fig. 1. Flow chart of patient selection.

HD settings

HD modalities were kept constant during the whole study period. All the sessions consisted of standard HD using standard dialysis solutions, with bicarbonate buffer. The dialyzer used was "Renak" 1.5 to 1.8 m^2. HD was performed in the regular setting in a quiet room. Adequacy of dialysis was assessed using KT/V, calculated as the natural logarithm of the ratio between initial and final urea concentration. Dialysate sodium concentration was the same for all the patients at 140 meq/l. The amount of UF was recorded for every patient in every session. Session UF rate was calculated

from the target UF volume at the start of each session (ml), per body weight (kg), per dialysis session length (h). Average UF rate for sessions delivered during the exposure period was calculated for each patient. No UF profile was used.

Data collection and description

Patient's demographic characteristics were obtained from the hospital electronic medical record, such as age, gender, dialysis vintage and initial nephropathy. The pulse pressure was calculated as the difference between the systolic and diastolic blood pressure for each pre- and post-dialysis session. Laboratory values and dialytic session characteristics taken during the study period were considered as the mean value measured during the exposure period. Prescribed antihypertensive medications were also considered, and it was not changed for the purposes of the study. The number of IHO episodes in 36 consecutive HD sessions was recorded for each patient. When hypotensive episodes occurred, the responsive measures included saline administration or premature cessation of dialysis.

Categorization of groups for analysis

The study patients were divided into two groups according to average SBP variation between post- and pre-dialysis values during 36 consecutive HD sessions: IHO group included patients who had their post-dialysis SBP dropped under 100 mm Hg with clinical symptoms during HD, for at least 50 % of HD sessions; Normotensive (NT) group included patients who did not have a major change in average post- and pre- dialysis SBP.

Plasma biochemical assays

During the study period, immediately before and at the end of each HD session, whole blood samples were collected in EDTA tubes, directly from vascular access. Whole blood was centrifuged at 4,500 RPM for 10 min. Plasma were aliquoted and stored at -80 °C for plasma biochemical assays to avoid freeze-thaw cycles.

The concentrations of different biochemical molecules were measured before and after HD sessions using enzyme-linked immunosorbent assay (ELISA) kits for NT and IHO groups, following the instructions of the manufacturers. No significant cross-reactivity or interference was observed in any kit.

Tumor necrosis factor α (TNF-α), Vascular Endothelial Growth Factor C (VEGF-C), endothelin 1 (ET-1), angiotensin converting enzyme type I (ACE I), aldosterone and renin ELISA kits were provided from R&D Systems Kits (R&D system Inc. Minneapolis, MN, USA) with a detection range: 0.5-5.5 pg/ml, 4.0-48.4 pg/ml, 0.031-0.207 pg/ml, 0.008-0.051 ng/ml, 7.74-22.4 pg/ml and 0.769-14.8 pg/ml respectively; and intra- and inter-assay coefficients of variation: 5.2-7.4 %, 6.6-8.5 %, 4.0-7.6 %, 3.4-7.7 %, 3.4-12.1 % and 5.3-5.5 % respectively. Asymmetric dimethylarginine (ADMA) and L-Citrulline ELISA kits were provided by Immunodiagnostik (Immunodiagnostik AG, Bensheim, Germany) with a detection range: 0.26-0.64 μmol/l and 1.5 μmol/l respectively; and intra- and inter-assay coefficients of variation: 5.8-7.6 % and 1.3-3.0 % respectively. Angiotensin Converting Enzyme 2 (ACE 2) was provided by IBL (Immuno-Biological Laboratories, Inc. Minneapolis, MN, USA) with a detection range: 62.5-4,000 pg/ml. Collectrin was provided by Cusabio (Cusabio Biotech CO., LTD, Wuhan, Hubei, China) with a detection range 51-800 pg/ml, and intra- and inter-assay coefficients of variation less than 15 % and less than 15 %, respectively.

C-reactive protein (CRP) was delivered by Abcam (Abcam, Cambridge, UK) with a minimal detectable dose of 4 pg/ml; and intra- and inter-assay coefficients of variation 5-3 %.

Statistical methods

Categorical data were reported as percentages and were compared using Chi² test or Fisher exact test as appropriate. Shapiro-Wilk and Kolmogorov-Smirnov tests were used to assess significant departure of continuous variables from normality. For variables with non-Gaussian distribution, the median with its interquartile range was used. For variables not departing significantly from normality, mean ± standard deviation was reported. Ordinal variables were compared using Mann-Whitney U test. For non-repeated measures, the student *t*-test was used. A repeated-measure multivariable analysis of variance model (MANOVA) was used to assess the variation of the dependent variables (DVs) (plasmatic levels of the different markers, and clinical parameters: SBP, heart rate, temperature), with one within factor (time defined as either pre or post-dialysis), and one between factor (group, NT or IHO groups). The interaction group*time was prespecified in the model. Univariate F tests were then conducted for individual DVs at the 5 % level. Due to the non-random design and the unbalanced group sizes, type III sum of squares was

used. MANOVA was performed using SPSS v22 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.).

Results

Participant's characteristics and SBP classification

Thirty eight men and twenty seven women completed the study period, their baseline demographics are presented in Table 1. Among the 65 HD subjects included in the study, 53 patients were NT and 12 patients experienced IHO, representing 19 % of the study population. During the study period, 432 HD sessions in IHO group were examined for IHO episodes by comparing post-dialytic with pre-dialytic blood pressure measurements and by evaluating symptoms of hypotension. The average intradialytic variation in SBP calculated as the difference between post- and pre-dialysis shows a clear difference between the two groups, with -16.76±8.53 mm Hg among NT and -36.50±8.12 mm Hg among IHO. SBP decreases at post-dialysis in both groups, but was significantly lower in IHO compared to NT group (p (interaction)<0.001, Table 1).

Table 1. Analytic characterization and clinical patient's data.

Variables		**NT (n=53)**	**IHO (n=12)**	*p* **value**
Age (y)		74 (63-80)	76 (56.5-79)	0.910
Gender (M/W)		32/21	6/6	0.534
Dialysis vintage (y)		8.05 ± 4.07	10.83 ± 3.16	0.100
Session duration (h)		4.5 (4-5)	4.15 (4-4.26)	0.015
Vascular access (%)	*Fistula*	49 (92)	11 (91)	0.629
	Jugular catheter	4 (8)	1 (9)	0.533
Mean SBP	*Pre-dialysis*	136 ± 11.59	126 ± 12.4	p (time)<0.001
	Post-dialysis	119 ± 8.03	89 ± 3.32	p (group)<0.001 p (interaction)<0.001
Mean pulse pressure	*Pre-dialysis*	63.87 ± 10.06	55.54 ± 6.76	p (time)<0.001
	Post-dialysis	54.59 ± 8.78	29.56 ± 2.87	p (group)<0.001 p (interaction)<0.001
Nephropathy origin (%)				
Hypertension		20 (38)	1 (8)	0.085
Diabetes mellitus		15 (28)	2 (16)	0.494
Glomerulonephritis		7 (13)	2 (16)	0.667
Polycystic kidney disease		4 (8)	3 (25)	0.111
Chronic tubulo-interstitial nephritis		4 (8)	1 (8)	0.999
Unknown		3 (5)	3 (25)	0.071
Risk factors (%)				
Smoking		28 (53)	3 (25)	0.113
Dyslipidemia		27 (51)	7 (58)	0.754
Diabetes		16 (30)	3 (25)	0.999
Obesity		13 (24)	3 (25)	0.999
Sedentary lifestyle		14 (26)	1 (8)	0.267
Alcohol		4 (7)	2 (16)	0.305
Previous coronary artery disease		17 (32)	3 (25)	0.652
Stroke		2 (4)	0 (0)	0.333
Peripheral vascular disease		10 (19)	2 (17)	0.909
Antihypertensive medication (%)				
ARB		14 (26)	3 (25)	1.000
β blockers		32 (60)	9 (75)	0.737
CCB		34 (64)	5 (41)	0.523

α blockers	13 (24)	1 (8)	0.438
α, β blockers	2 (3)	2 (16)	0.152
Diuretics	19 (35)	0 (0)	0.013
Vasodilators	12 (22)	4 (33)	0.109
Combination of antihypertensive drugs (%)			
More than 2 combinations	28 (53)	4 (33)	0.263
Laboratory data			
Hematocrit (%)	32 ± 3.67	33.19 ± 4.40	0.347
Hemoglobin (g/l)	10.57 ± 1.24	10.93 ± 1.49	0.396
Alkaline phosphatase (U/l)	90 (76.5-123.0)	98 (62-155.75)	0.637
Serum glutamic pyruvic transaminase (IU/l)	24 (19-27)	24 (21-29.5)	0.623
Parathyroid hormone (pg/ml)	179 (127-275)	71.8 (54.1-443.5)	0.670
Serum bicarbonate (mmol/l)	21.92 ± 3.17	21.08 ± 2.11	0.386
Serum phosphorus (mmol/l)	1.56 ± 0.46	1.80 ± 0.48	0.118
Serum calcium (mmol/l)	2.26 ± 0.15	2.25 ± 0.12	0.694
Urea reduction ratio	0.75 ± 0.06	0.76 ± 0.09	0.537
Creatinine (μmol/l)	280.85 ± 98	299.55 ± 87	0.561

Values are represented as mean ± SD or as median with its interquartile range (IQR=Q1-Q3). The *t*-test, Mann-Whitney U test and Fisher exact test were used as appropriate. Two-way MANOVA for repeated measures is represented by 3 types of *p* values: *p* (Time) associated with the within subject effect (Time factor, 2 levels, pre and post-dialysis), *p* (Group) associated with the between subject's effect (Group factor, 2 levels, NT and IHO groups), and the *p* (Interaction) associated with the group*time interaction term. NT: normotensive; IHO: Intradialytic Hypotension; SBP: systolic blood pressure; ARB: angiotensin II receptor blocker; CCB: calcium channel blockers; n: number of patients in each group, y: years.

The demographic parameter did not differ significantly between the two groups (Table 1). The mean age was 74 (63-80) in NT group and 76 (56.5-79) in IHO group, median time on HD was 8.05±4.07 years in NT group, and 10.83±3.16 years in IHO group. Women comprised 65 % of the NT group and 50 % of IHO group. Hypertensive nephropathy was the most frequent cause of end stage kidney disease in NT group (38 %), while in IHO group it was the polycystic kidney disease (50 %). The catheter rate was the same in both groups (Table 1). The majority of patients were on antihypertensive medications, calcium channel blockers (CCB) and β blockers were the most commonly prescribed. Laboratory measurements show no difference between the two groups (Table 1).

Analytical measurements

Dry weight was determined clinically with the help of echocardiography when needed by assessing the inferior vena cava diameter. Interdialytic weight gain and Intradialytic weight loss were comparable in both groups (Table 2), leaving the patients at their presumed dry weights. The body temperature and heart rate were comparable during HD sessions in both group (Table 2). Pre- and post-dialysis Na plasma concentrations were similar in both groups (Table 3). Serum K concentration followed the same progression in both groups, but was slightly higher in IHO group. UF rate was comparable between the two groups (Table 3). To determine the effect of UF, univariate Spearman's correlation was used to determine whether there were any significant associations between intradialytic SBP variation during HD sessions and UF rate in both groups. There was no direct association between change in SBP and UF rate in NT (r=0.164, *p*=0.240, n=53, Fig. 2A) and IHO groups (r=-0.231, *p*=0.456, n=12, Fig. 2B).

RAAS markers assessment

RAAS was evaluated by plasma concentration measurements of renin, ACE I, aldosterone and ACE 2. Comparing post- to pre-dialysis, renin, ACE I and ACE 2 plasma concentrations significantly increased in NT and IHO groups (*p* (time)<0.001, *p* (time)<0.001 and *p* (time)<0.001 respectively, Fig. 3), with higher levels in NT group compared to IHO group for renin (*p* (interacttion)<0.001, Fig. 3) and ACE I (*p* (interaction)=0.002, Fig. 3) plasma concentrations. However, post-dialysis ACE 2 plasma concentration was significantly higher in IHO group compared to NT group (*p* (interaction)=0.001, Fig. 3). Aldosterone plasma concentration increased significantly in the NT group while it decreased in the IHO group (*p* (interaction)<0.001, Fig. 3).

Table 2. Pre- and post-dialysis clinical parameters measurements during each HD session over the study period.

Parameters		NT	IHO	p value
Mean body weight (kg)	Pre-dialysis	74.8 ± 17.7	75.16 ± 13.94	0.945
	Post-dialysis	71.9 ± 17.2	72.70 ± 13.26	0.884
Mean Intradialytic weight loss (% Intradialytic weight loss)		2.86 ± 0.88 (3.87 ± 1.01)	2.51 ± 1.00 (3.29 ± 0.96)	0.225 (0.075)
Mean Interdialytic weight gain (% Interdialytic weight loss)		2.80 ± 0.99 (3.95 ± 1.28)	2.51 ± 0.93 (3.46 ± 1.12)	0.348 (0.220)
Mean body temperature (°C)	Pre-dialysis	35.9 ± 0.3	35.97 ± 0.28	p (time)=0.090 p (group)=0.885 p (interaction)=0.553
	Post-dialysis	36.1 ± 0.63	36.09 ± 0.27	
Mean heart rate (beats/min)	Pre-dialysis	72 ± 8.67	72.70 ± 9.41	p (time)=0.034 p (group)=0.620 p (interaction)=0.549
	Post-dialysis	73 ± 7.49	74.29 ± 8.59	

Values are represented as mean ± SD. The student t-test was used for non-repeated measures. The Two-way MANOVA test was used for repeated measures, and is represented by 3 types of p values: p (Time) associated with the within subject effect (Time factor, 2 levels, pre and post-dialysis), p (Group) associated with the between subject's effect (Group factor, 2 levels, NT and IHO groups), and the p (Interaction) associated with the group*time interaction term. NT: normotensive; IHO: Intradialytic Hypotension.

Table 3. Biological forum and UF rate in NT and IHO groups.

Markers		NT	IHO	p value
ET-1 plasma concentrations				
ET-1 (pg/ml)	Pre-dialysis	0.58 ± 0.29	2.48 ± 1.63	p (time)=0.840 p (group)<0.001 p (interaction)=0.003
	Post-dialysis	1.01 ± 0.32	2.02 ± 1.21	
Electrolytes				
Serum Na (mmol/l)	Pre-dialysis	138.70 ± 3.47	139.67 ± 3.92	p (time)<0.001 p (group)=0.652 p (interaction)=0.495
	Post-dialysis	143.60 ± 2.06	143.55 ± 2.42	
Serum K (mmol/l)	Pre-dialysis	5.34 ± 0.63	5.97 ± 0.76	p (time)<0.001 p (group)=0.237 p (interaction)=0.011
	Post-dialysis	3.43 ± 0.29	3.27 ± 0.24	
Inflammation markers plasma concentrations				
CRP (mg/l)	Pre-dialysis	17.61 ±7.44	21.24 ±7.60	p (time)=0.003 p (group)=0.216 p (interaction)=0.433
	Post-dialysis	18.11 ± 8.03	22.76 ± 2.02	
TNF-α (pg/ml)	Pre-dialysis	12.90 ± 2.49	13.36 ± 4.37	p (time)=0.486 p (group)=0.064 p (interaction)=0.959
	Post-dialysis	14.40 ± 3.78	17.25 ± 5.55	
UF rate				
UF rate index (ml/h/kg)		12.99 ± 3.17	13.00 ± 1.53	0.866
Erythropoietin				
Erythropoietin (UI/kg/week)		65.68 ± 14.4	78.07 ± 30.69	0.239

Values are represented as mean ± SD. The student t-test was used for non-repeated measures. Two-way MANOVA for repeated measures is represented by 3 types of p values: p (Time) associated with the within subject effect (Time factor, 2 levels, pre and post-dialysis), p (Group) associated with the between subject's effect (Group factor, 2 levels, NT and IHO groups), and the p (Interaction) associated with the group*time interaction term. NT: normotensive; IHO: Intradialytic Hypotension; RAAS: renin-angiotensin-aldosterone system; ACE I: Angiotensin Converting Enzyme type I; ACE 2: Angiotensin Converting Enzyme type 2; Na: sodium; K: potassium; ET-1: Endothelin 1; CRP: C-reactive protein; TNF-α: Tumor Necrosis Factor α; UF: ultrafiltration.
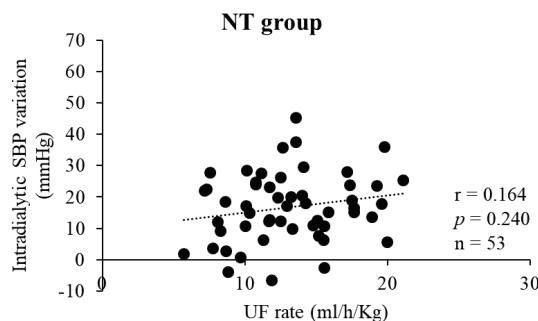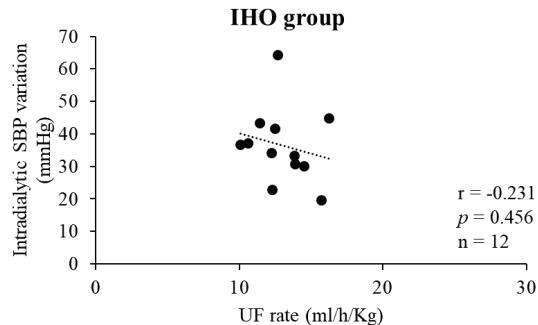
Fig. 2. Correlation between intradialytic SBP variation and UF rate in NT (**A**) and IHO (**B**) groups. Spearman's rank correlation (r=0.164, *p*=0.240, n=53) for NT group, and (r=-0.231, *p*=0.456, n=12) for IHO group. NT: normotensive; IHO: Intradialytic Hypotension; SBP: systolic blood pressure; UF: ultrafiltration.
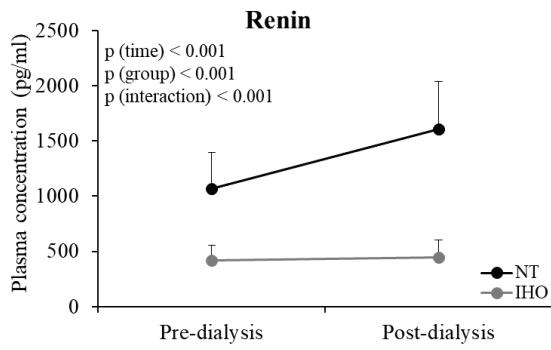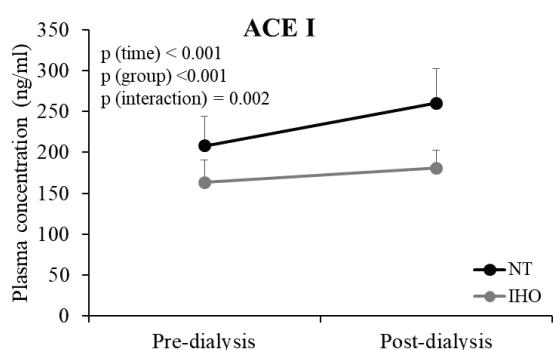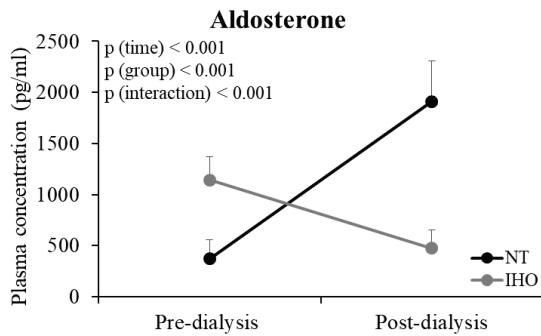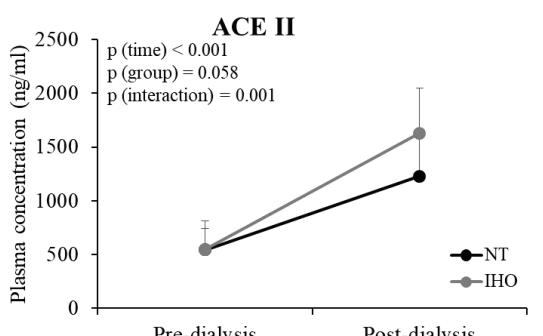

Fig. 3. RAAS evaluation in NT and IHO groups. Intradialytic changes of mean plasma concentrations of (**A**) Renin, (**B**) ACE I, (**C**) Aldosterone and (**D**) ACE 2 were measured between pre- and post-dialysis in NT and IHO groups. Values are represented as mean ± SD. All the data were evaluated by the Two-way MANOVA for repeated measures and represented by 3 types of *p* values: *p* (Time) associated with the within subject effect (Time factor, 2 levels, pre and post-dialysis), *p* (Group) associated with the between subject's effect (Group factor, 2 levels, NT and IHO groups), and the *p* (Interaction) associated with the group*time interaction term. NT: normotensive; IHO: Intradialytic Hypotension; ACE I: angiotensin converting enzyme type I; ACE 2: Angiotensin Converting Enzyme 2.

Inflammation markers

Inflammatory markers were assessed by measuring CRP and TNF-α plasma concentrations; they increased in post-dialysis in both groups (*p* (time)=0.003, Table 3), no differences were detected between groups for CRP (*p* (interaction)=0.433, Table 3), and for TNF-α (*p* (interaction)=0.959, Table 3) plasma concentrations.

ET-1, NO synthesis and VEGF-C plasma concentration assessment

Pre- and post-dialysis ET-1 plasma concentration increased significantly in IHO group compared to NT group (*p* (group)<0.001, *p* (interaction)=0.003, Table 3). NO synthesis control involves ADMA (NO synthase inhibitor) and L-Citrulline which is secreted in equimolar amounts to NO and Collectrin

(L-Arginine uptake enhancer). Post-dialysis L-Citrulline plasma concentration significantly decreased in both groups (*p* (time)<0.001, Fig. 4A). Post-dialysis ADMA plasma concentration was significantly higher in IHO group compared to NT group (*p* (group)=0.001, Fig. 4B). Whereas, pre- and post-dialysis Collectrin plasma concentrations were significantly lower in IHO group compared to NT group (*p* (time)<0.001,

p (group)=0.002, p (interaction)=0.057, Fig. 4C). In NT group, VEGF-C plasma concentration slightly increased in post-dialysis compared to pre-dialysis, while in IHO group, the increase was more pronounced (p (time)<0.001, Fig. 4D). Post-dialysis VEGF-C plasma concentration significantly increased in IHO group compared to NT group.
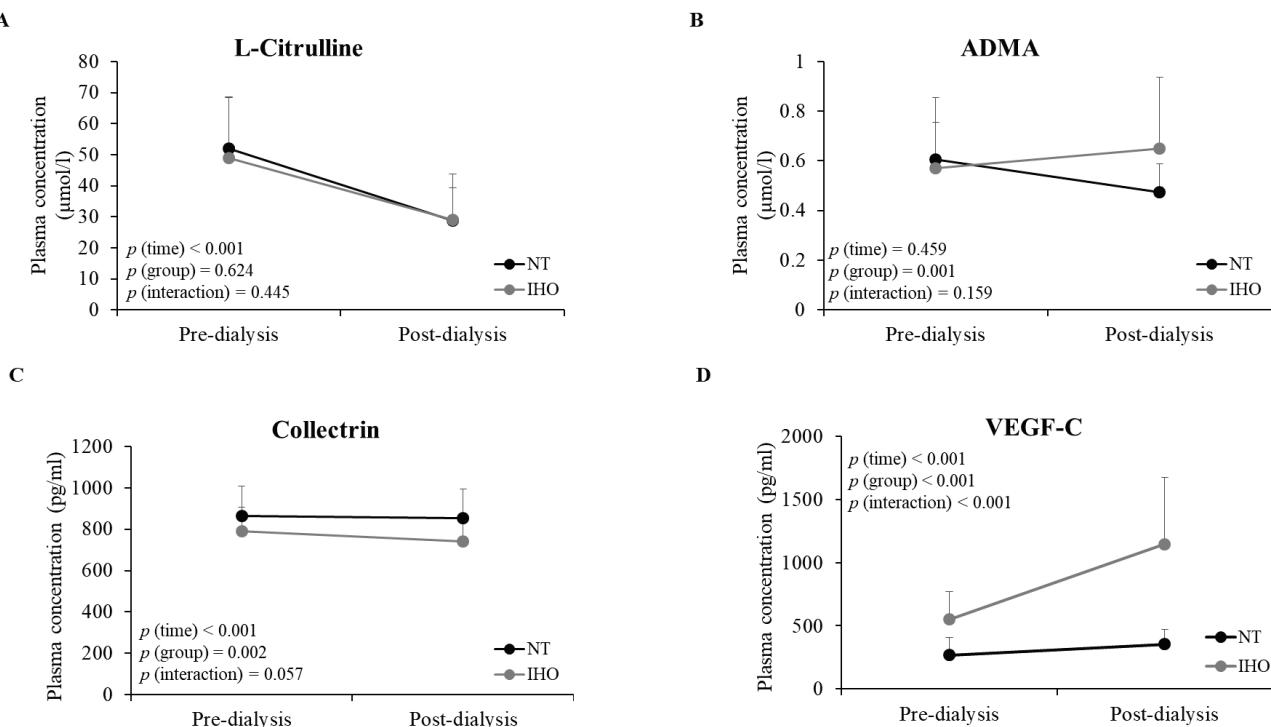
Fig. 4. ADMA, collectrin, VEGF-C and L-Citrulline plasma concentrations in NT and IHO groups. Intradialytic changes of mean plasma concentrations of (**A**) L-Citrulline, (**B**) ADMA, (**C**) collectrin and (**D**) VEGF-C were measured between pre- and post-dialysis in NT and IHO groups. Values are represented as mean ± SD. All the data were evaluated by the Two-way MANOVA for repeated measures and represented by 3 types of *p* values: *p* (Time) associated with the within subject effect (Time factor, 2 levels, pre and post-dialysis), *p* (Group) associated with the between subject's effect (Group factor, 2 levels, NT and IHO groups), and the *p* (Interaction) associated with the group*time interaction term. NT: normotensive; IHO: Intradialytic Hypotension; ADMA: Asymmetric Dimethylarginine; VEGF-C: Vascular Endothelial Growth Factor type C.

Discussion

Hemodynamic status during HD is subject to multifactorial process related to the HD procedure itself and/or to the patient's profile. IHO is known to be associated with cardiovascular complications and all-cause of mortality (Flythe *et al.* 2015). Thus, an awareness of the pathophysiology of IHO may help to identify patients who are most susceptible to experience IHO episodes during HD and to prevent its onset. Endothelial impairments constitute a key element in blood pressure control (Dubin *et al.* 2011). Indeed, an imbalance between vasoconstrictor and vasodilator

agents could be responsible in part of the occurrence of IHO. Therefore in this study, we focused on the endothelial micro-environment biomarkers in sixty five patients from the tertiary university hospital Hotel Dieu de France, Beirut.

Excess UF is a known cause of IHO (Flythe *et al.* 2011, Ok *et al.* 2016). The removal of extracellular fluid by UF procedure results in 10-30 % decrease in blood volume during HD session duration. IHO occurs when plasma volume falls below a critical threshold (Kooman *et al.* 2007, Ok *et al.* 2016). High UF rate is associated with excess mortality due to cardiovascular complications (Assimon *et al.* 2016). Few studies have

demonstrated a relationship between the blood volume and the development of IHO (Reeves and Mc Causland 2018), whereas many others disapproved this relation (Krepel *et al*. 2000, Tonelli *et al*. 2002, Reeves and Mc Causland 2018), suggesting that other (poorly understood) factors could be involved (Dasselaar *et al*. 2009). Moreover, a high percentage of IHO episodes was shown to occur during isovolumic dialysis or without UF (Wehle *et al*. 1979, Dheenan and Henrich 2001). In our study population, the dry weight was frequently assessed and adjusted, moreover, mean UF rate / dry weight was comparable between IHO and NT groups (13.00±1.53 ml/h/kg vs. 12.99±3.17 ml/h/kg respectively). This suggests that UF rate alone cannot account for IHO episodes.

The majority of our patients were on antihypertensive medications, this could affect the occurrence of IHO, however the similarity of the pre-dialysis blood pressure between both groups and the higher number of antihypertensive medications per patient in the NT group are not in favor of this theory. Moreover, many studies previously addressed this issue, and did not find a correlation between antihypertensive medication used and the occurrence of IHO (Chang 2017).

Tachycardia in response to hypotension did not occur maybe because of the use of β blockers drugs or because of autonomic dysfunction, frequent in this population of patients.

Blood pressure regulation depends intimately on the RAAS and the endothelial vasoactive agents balance. Plasma renin, ACE I and aldosterone concentrations were shown to increase in hypotensive dialyzed patients (Moore *et al*. 1989, Landry and Oliver 2006) or to be compromised (Reeves and Mc Causland 2018). In our study, the more pronounced increase in post-dialysis renin, ACE I and aldosterone plasma concentrations in NT compared to IHO group suggest an impairment of the RAAS response to hypotension in the IHO group. Regarding the higher level of pre-dialysis aldosterone in the IHO group, it may be explained by higher plasma potassium concentration (Schambelan *et al*. 1980, Sinha and Agarwal 2013).

ACE 2 is identified as a novel actor in the RAAS (Donoghue *et al*. 2000). The most-important active product of ACE 2 is Ang (1-7), which can be primarily generated *via* Angiotensin II direct hydroxylation to produce Ang (1-7), or *via* transformation of angiotensin I to angiotensin 1-9, which is then cleaved to produce Ang

(1-7) (Jiang *et al*. 2014). Ang (1-7) has broad effects in the cardiovascular system, including vasodilatation and antihypertensive effects (Sampaio *et al*. 2007). ACE 2 plasma concentration increases in both groups, but in a more marked manner in post-dialysis IHO group, suggesting a role for ACE 2 in inducing vasodilation and hypotension in affected patients.

Endothelial vasoactive agents, particularly ET-1, play an important role in the maintenance of hemodynamic stability during HD. Many studies found unchanged or moderate increase ET-1 plasma levels during HD (Miyauchi *et al*. 1991, Miyauchi *et al*. 2012, Deray *et al*. 1992), while others found a decrease (Raj *et al*. 2002, El-Shafey *et al*. 2008). In the present study, plasma ET-1 concentrations showed higher values in post-dialysis IHO group. This could be explained as a compensatory response to the fall of blood pressure.

Even more, an imbalance between NO and ET-1 may affect the development of IHO. NO/ET-1 ratio is a strong predictor of hemodynamic consequence (Raj *et al*. 2002). NO is a powerful vasodilator, produced by endothelial cells (Amore *et al*. 1995, Tousoulis *et al*. 2012). The plasma concentration of NO is difficult to measure because of his short half-life (10-30 s), and it diffuses rapidly through all cell membranes (Furchgott and Vanhoutte 1989). Nevertheless measuring L-Citrulline which is secreted simultaneously and in equal quantity with NO can be a good tool. In our study, L-Citrulline plasma concentrations are comparable between the two groups in pre- and post-dialysis.

In addition, changes in the plasma concentration of NO inhibitors are also involved in maintaining hemodynamic stability during HD. ADMA is an endogenous competitive inhibitor of NO synthase, and thus may cause endothelial dysfunction (Sibal *et al*. 2010). Increase in ADMA plasma concentration is associated with angiogenic disorders in hypercholesterolemia, insulin resistance, hypertension and homocysteinemia (Cooke 2003) and an abnormal endothelial regeneration *in vitro* (Azuma *et al*. 1995). ADMA is released as a result of proteolysis of proteins containing methylarginine residues. ADMA is hydrolyzed to Citrulline and methylamines by dimethylarginine dimethylaminohydrolases I and II (Vallance and Leiper 2004). A positive correlation was observed between the decrease of ADMA concentration and the occurrence of intradialytic hypotensive episodes (Vallance *et al*. 1992, Raj *et al*. 2002, Hewitson *et al*. 2007). Herein, ADMA plasma concentration increased with HD in our

population study. This result can be a compensatory response to the lack of L-Arginine and to avoid further decrease in SBP in this specific group.

Collectrin is a trans-membrane protein expressed in the collecting duct and in the vascular endothelium of conduit and resistance vessels. Collectrin has a role in facilitating the cellular uptake of L-Arginine, substrate of endothelial NO synthase to produce NO (Chu and Le 2014). Herein, Collectrin plasma concentration decreased during HD in both groups, but more markedly in IHO group, resulting in a significant decrease in L-Arginine bioavailability and therefore a decrease in NO synthesis (Cechova *et al.* 2013). Our results suggest that the decrease of NO release may be a compensatory mechanism trying to protect patient from further dramatic decrease in blood pressure. To the best of our knowledge, this is first study that link collectrin to IHO episodes.

Another important endothelial cell factor is the circulating VEGF-C (Machnik *et al.* 2009). VEGF-C is essential for endothelial cell growth, cell differentiation, angiogenesis and lymphangiogenesis (Zachary and Gliki 2001, Lahdenranta *et al.* 2009). Recently, VEGF-C was showed to be implicated in hypervolemia in chronic kidney disease (Sahutoglu *et al.* 2017). Here, this is the first study to relate VEGF-C to IHO physiopathology.
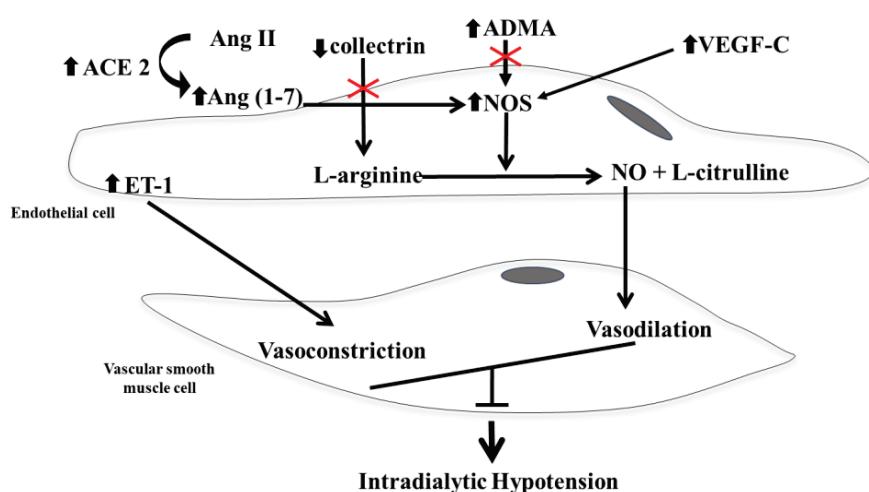
The higher VEGF-C concentrations observed in post-dialysis in the IHO group may contribute to the development of IHO by enhancing lymphatic drainage of salt inducing a decrease in blood pressure.

In an effort to combine the results and explain the role in IHO genesis, we elaborated a unifying scheme summarizing the explored interactions and pathways (Fig. 5).

As a matter of course, the current study is limited by the sample size, the single facility design and the short duration of follow up. Despite its limitations, the current study is to date the first to assess collectrin and VEGF-C in IHO patients.

In conclusion, the study generated several interesting hypothesis to be considered for future investigations, where endothelial microenvironment dysregulation and VEGF-C impairment may contribute to the pathogenesis of IHO.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

Fig. 5. Proposed scheme summarizing the effect of the studied molecules on IHO pathogenesis. The ACE 2 increase results in Ang (1-7) production which in turn enhances NOS activation and therefore more NO production, despite the decrease of Collectrin. Collectrin decrease, leads to less L-Arginine bioavailability associated with a compensatory increase of ADMA and consequent inhibition in NO synthesis. In parallel, VEGF-C activates NOS. All in all, the vasomotor net effect is going toward more vasodilation status.

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