Health Sciences & Disease
The Journal of Medicine and Biomedical Sciences

Article Original

Variation of Serum Bilirubin in Chronic Kidney Disease Cameroonian Patients on Maintenance Haemodialysis

Taux de bilirubine sérique chez les patients atteints de maladie rénale chronique avant et après l'hémodialyse

Nzana Victorine B[1,2*], Maimouna Mahamat [1,3], Maheg André B[4], Kowo Mathurin [1,5], Kaze Francois[1,5], Ama Moor Vicky[4,5]

Affiliations
[1] Department of Internal Medicine and Specialties, Faculty of Medicine and Biomedical Sciences, University of Yaounde 1, Yaounde, Cameroon
[2] Yaounde Central Hospital
[3] Yaounde General Hospital
[4] Department of Biochemistry, Faculty of Medicine and Biomedical Sciences, University of Yaounde 1, Yaounde, Cameroon
[5] Yaounde University Teaching hospital, Yaounde, Cameroon.

[*]**Correspondence**: NZANA Victorine B; vickybandolo@yahoo.fr

Keywords: haemodialysis, serum total and direct bilirubin
Mots clés : hémodialyse, bilirubine sérique totale et direct.

ABSTRACT

Background. Bilirubin has potent antioxidant properties and higher bilirubin levels even within normal range are associated with beneficial effects in HD patients. However, the presence of oxidative stress may deplete antioxidants including serum bilirubin. In this work, we sought to evaluate the serum bilirubin levels in chronic kidney disease patients before and after haemodialysis. **Methods**. We carried out a 9 months cross sectional study in adults patients on MHD for at least three months at the Yaoundé University Teaching hospital. Socio-demographic, clinical and para-clinical data were collected. Blood samples for serum bilirubin were collected before and after HD sessions. Measurement of serum bilirubin was done using spectrophotometer. The association between the variables of interest was evaluated with the Mann Whitney and Pearson chi-square tests of correlation with a significant statistical level, P<0.05. **Results.** We included 60 participants (68.3% males) with a mean age of 48.7±13.6 years. The median dialysis vintage was 14.0 [6.0-32.5] months with 90% using arterio-venous as vascular access. The median values [IQR] of total bilirubin and direct bilirubin before HD were 4.4 [03.2 - 06.4] mg/l and 1.5 [01.2 - 01.9] mg/l and after HD, were 5.2 [03.6 - 08.2] mg/l and 1.6 [01.4 - 02.1] mg/l respectively. **Conclusion.** Serum bilirubin increases after haemodialysis due to haemoconcentration though serum levels may be depleted by the presence of oxidative stress often presence in haemodialysis patients

RÉSUMÉ

Introduction : la bilirubine possède de puissantes propriétés antioxydantes et des taux élevés de bilirubine, même dans les limites de la normale, sont associés à des effets bénéfiques chez les patients atteints de la maladie rénale chronique. Cependant, la présence d'un stress oxydatif peut épuiser les antioxydants, y compris la bilirubine sérique. Notre objectif était d'évaluer les taux de bilirubine sérique chez les patients atteints de maladie rénale chronique avant et après l'hémodialyse. **Méthodes.** Nous avons mené une étude transversale de 9 mois chez des adultes en hémodialyse (HD) depuis au moins trois mois au CHU de Yaoundé. Les données sociodémographiques, cliniques et paracliniques ont été recueillies. Des échantillons de sang pour la bilirubine sérique ont été prélevés avant et après les séances d'HD. La mesure de la bilirubine sérique a été effectuée à l'aide d'un spectrophotomètre. L'association entre les variables d'intérêt a été évaluée avec les tests de corrélation de Mann Whitney et du chi-carré de Pearson avec un niveau statistique significatif, P<0,05. **Résultats.** Nous avons inclus 60 participants (68,3% d'hommes) avec un âge moyen de 48,7±13,6 ans. L'ancienneté médiane de la dialyse était de 14,0 [6,0-32,5] mois avec 90% utilisant l'artério-veineux comme accès vasculaire. Les valeurs médianes [IQR] de la bilirubine totale et de la bilirubine directe avant HD étaient respectivement de 4,4 [03,2 - 06,4] mg/l et 1,5 [01,2 - 01,9] mg/l et après HD de 5,2 [03,6 - 08,2] mg/l et 1,6 [01,4 - 02,1] mg/l. **Conclusion.** La bilirubine sérique augmente après l'hémodialyse en raison de l'hémoconcentration, bien que les niveaux sériques puissent être réduits par la présence d'un stress oxydatif souvent présent chez les patients hémodialysés.

HIGHLIGHTS

What is already known on this topic

Bilirubin has potent antioxidant properties and higher bilirubin levels even within normal range are associated with beneficial effects in haemodialysis (HD) patients. However, the presence of oxidative stress may deplete antioxidants including serum bilirubin.

What question this study addressed

Serum bilirubin levels in chronic kidney disease Cameroonian patients before and after HD.

What this study adds to our knowledge

Serum bilirubin increases after HD due to haemoconcentration though serum levels may be depleted by the presence of oxidative stress.

How this is relevant to practice, policy or further research.

Further studies are needed to confirm our findings.

INTRODUCTION

Haemodialysis (HD) is the commonest form of kidney replacement therapy in the world, accounting for approximately 69% of all kidney replacement therapy and 89% of all dialysis. Patients on maintenance HD show peculiar features of clinical presentation and diseases including cardiovascular, metabolic, hematologic, and liver diseases. As a catabolic end-product of haem, bilirubin has potent antioxidant properties and is associated with mortality rate in HD patients. Bilirubin increases in both cholestatic and hepatotoxic liver disease. The role that serum total bilirubin (STB) within the physiological range, plays in the development and progression of kidney disease remains controversial[1]. Several studies have suggested that an inverse association between STB and the progression of ESRD[2, 3, 4, 5] plays a potential protective role in renal outcomes. A large study of the Korean population that was recently published demonstrates that individuals with higher bilirubin levels have a reduced prevalence of CKD originating from diabetes in women[6]. Two studies revealed that elevated bilirubin levels have a reduced risk of progressing from urinary micro albuminuria to macro albuminuria, as well as improved eGFR in diabetic patients[7, 8]. Furthermore, Fukui *et al.* reported that higher circulating serum bilirubin levels were associated with reduced risk of cardiovascular disease and mortality in dialysis patients [9]. A higher serum bilirubin concentration in physiological ranges is associated with a lower risk for the development and progression of both chronic kidney disease (CKD) and cardiovascular disease (CVD) in adults. The protective mechanisms of bilirubin in CVD, CKD, and associated mortality may be ascribed to its antioxidant and anti-inflammatory properties[10]. However Wang *et al.* reported that lower STB was not an independent protective factor in kidney disease progression among hypertensive patients who never smoke[11]. Targher *et al.* demonstrated that higher STB levels were significantly associated with lower eGFR in both non-diabetic and diabetic individuals in unselected outpatients[12]. Ryu *et al.* proved that neither STB nor indirect bilirubin levels were associated with the incidence of CKD[13]. Additionally, it was noted that in patients with ESRD who were undergoing haemodialysis, high concentrations of bilirubin were correlated with a higher mortality rate[9]. High total bilirubin levels above physiologic range is associated with mortality in CKD patients undergoing long-term HD[9]. Little is known about the variation of bilirubin level with HD, hence we sought to evaluate the serum bilirubin levels in chronic kidney disease patients before and after haemodialysis.

METHODS

We carried out a cross-sectional study at the haemodialysis unit of the Yaounde University Teaching Hospital for a period of 9 months from January 2022 to September 2022. We included consenting patients who were at least 18 years old and who were undergoing maintenance HD for more than 3 months. We excluded from our study population, patients who were fasting and those whose blood samples were not taken before and after HD. Ethical clearance (Ref: N° 442/UYI/FMSB/VDRC/DAAR/CSD) was obtained from the institutional ethical review board of the Faculty of Medicine and Biomedical Sciences (FMSB) of the University of Yaounde I. We used the HD register to identify patients and their dialysis schedule. For each eligible patient, we presented our study as he/she came for HD and obtained consent. For each consenting participant, we verified that they were not fasting, we interviewed them, and then we reviewed their medical records for relevant socio-demographic and clinical data. Our variables of interest were socio-demographic data (age, sex), Clinical data (Comorbidities, aetiology of CKD, haemodialysis vintage and ongoing treatment). Thereafter we conducted a physical exam and collected 5 ml of each patient's blood in a dry tube for the biological analysis using a 10ml syringe sample before and after HD. Collected blood samples were stored in a cooler at a temperature of + 8ºC and far from the reach of Ultraviolet light (due to the fact that bilirubin being photo labile) and samples were transported to the laboratory within 4hours. The samples were centrifuged at 3000 rpm for 6 minutes and 1500µl (1.5ml) of serum was collected using a 1000µl micropipette and placed in cryovials for biochemical assays. This principle of the biochemical assay of Total and Direct Bilirubin was based on a reaction between bilirubin and diazotized sulfanilic acid which leads to a compound, the azobilirubin, coloured in very acid or basic medium. In this principle modified by Walters et al, bilirubin reacts in aqueous solution. In order to assay total bilirubin, the link between unconjugated bilirubin and albumin was broken down by adding dimethyl sulfoxide. The absorbance of azobilirubin produced was proportional to the concentration of bilirubin and was measured at 550nm (530-580). Manually, we placed reagents and specimen at room temperature. We started our timer after adding and mixing the specimen. We first read all the blanks of one run then read all the assays and

drained the cuvette between each tube. Absorbances (Abs.) were read at 550nm (530-580) against blanks. Total bilirubin values were read after more than 3 minutes at 37°C on spectrophotometer. Direct bilirubin values were read at exactly 3 minutes at 37°C on spectrophotometer. We obtained our bilirubin concentrations as follows: Results in mg/dL= [Abs. blank -Abs. assay] ×114

Table I: procedure for total and direct bilirubin

	Assay	Blank
Reagent r1 (total bilirubin)/ r2(direct bilirubin)	**1000µl**	**1000µl**
Distilled water		50µl
Reagents r3	50µl	
We added specimen, calibrator, and control serums.	100µl	100µl
µL= microliter		

RESULTS

A total of 62 patients were recruited, among whom, 2 were excluded for unavailability of blood samples after HD. Of the 60 patients enrolled, 68.3% were males and the mean age was 48.7±13.6 years. As baseline nephropathies, chronic glomerulonephritis, hypertensive nephropathy and diabetic nephropathy were the most frequent with prevalences of 28.3%, 25% and 23.3% respectively. High blood pressure (HBP) was the most prevalent (n=55, 91.7%) with 87.3% on antihypertensive treatment. The median dialysis vintage was 14.0 [6.0-32.5] months with a 90% (n=54) using arterio-venous fistulas as vascular access. Of the 15 patients who were diabetic, 73.3% were on anti-diabetic treatment while all the 8 HIV positive patients were on combined antiretroviral therapies. Two patients had viral hepatitis C and none had viral hepatitis B. Six patients consumed alcohol and 1 patient was a smoker.

Table II: drug history of the study population

Variable	N	%
Antihypertensive treatment(n=55)		
Yes	48	87.3
No	07	12.7
Type of antihypertensive drugs		
Calcium channel blockers	41	85.4
Diuretics	21	43.7
ACE inhibitors	11	22.9
Methyldopa	06	12.5
Anti-diabetic treatment (n=15)		
Yes	11	73.3
No	04	26.7
cART treatment (n=8)		
Yes	08	100.0
No	00	0.0

cART= combination antiretroviral treatment, ACE inhibitors= Angiotensin converting enzyme inhibitors

The median values of total bilirubin and direct bilirubin before HD were 4.4 [03.2 - 06.4] mg/l and 1.5 [01.2 - 01.9] mg/l and after HD, these values were 5.2 [03.6 - 08.2] mg/l and 1.6 [01.4 - 02.1] mg/l respectively.

Table III: comparison of data results before and after HD in the study population (N=60)

Variable	Before HD Median [P_{25} – P_{75}]	After HD Median [P_{25} – P_{75}]	p Value
Total Bilirubin (mg/L)	4.4 [03.2 - 06.4]	5.2 [03.6 - 08.2]	0.002
Direct Bilirubin (mg/L)	1.5 [01.2 - 01.9]	1.6 [01.4 - 02.1]	0.173
Indirect Bilirubin (mg/L)	2.8 [02.0 - 04.6]	3.7 [02.4 - 06.2]	0.002

P25= lower quartile, P75= higher quartile; IU= International unit; mg= milligrams; L= liters

DISCUSSION

To the best of our knowledge, this study is amongst the few in sub-Saharan Africa on the variation of serum bilirubin in Haemodialysis patients. However, our population may not be representative of patients on maintenance haemodialysis in Cameroon as we included only the haemodialysis population of a single unit in the centre region.

This was a cross-sectional analytical study conducted in a population of chronic kidney disease patients on maintenance haemodialysis in which we determined their serum bilirubin levels before and after haemodialysis. The mean age in the study population was 48.7±13.6 years which is similar to that of Liberato *et al.*, who had a mean age of 54.0±15.0 years[6]. This finding reflects the mean age of patients on haemodialysis in Africa and in Brazil, where the majority of haemodialysis population are in the age group 45-64 years old[14]. Males accounted for 68.3% with a sex ratio (male/female) of 2.16:1. These findings are similar to those of *Halle et al.*, in 2016 who had a male predominance of 1.98 in sub-Sahara Africa[15]. Furthermore, this male predominance in haemodialysis is noted globally, especially where there is no healthcare insurance[16]. This could be explained by the fact that, in the absence of health insurance, health care is out of pocket and most men are the bread winners, hence they will turn to prioritize themselves. The main baseline nephropathies were chronic glomerulonephritis, diabetic and hypertensive nephropathies similar to the results obtained by Halle *et al.*, in 2015 in Cameroon[17] and they remain the main aetiologies reported worldwide. Our median serum total bilirubin level was 4.4 [03.2-06.4] mg/l is lower than the mean serum total bilirubin level of 7.0± 2.7mg/l reported by Sudha *et al*[18]. Our lower value could be explained by pronounced oxidative stress due to retention of a plethora of toxins, subsidized under uraemia since our patients barely do twice weekly HD with several interruptions such as lack of consumables, leading to plasma depletion of serum bilirubin. Furthermore patient nutrition may lack antioxidants which may aggravate the depletion of plasma bilirubin. The median values of total serum bilirubin significantly increased after HD, with values before and after HD being 4.4 [03.2-06.4] mg/l and 5.2[03.6-08.2] mg/L respectively. Similarly the median indirect bilirubin levels increased significantly from 2.8

[02.0 - 04.6] to 3.7 [02.4 - 06.2]. Our findings are similar to those of Tian *et al.*, who reported a median indirect bilirubin value of 4.8 [3.3-7.0] µmole/L[19]. Our finding could be attributed to haemoconcentration. Bilirubin is a potent antioxidant and said to affect atherosclerosis by inhibiting low density lipoprotein oxidation, vascular smooth muscle cell proliferation and endothelial dysfunction[20]. Recently, higher bilirubin levels are associated with beneficial effects in HD patients[21]. Clinical studies have shown that higher levels of serum bilirubin, even within normal range, are associated with reduced all-cause mortality. Dialysis removes water-soluble circulating antioxidants, including uric acid and ascorbate, but does not remove hydrophobic substances, such as unconjugated bilirubin, which is plasma albumin-bound[5]. Duration of dialysis therapy, iron infusion, anaemia, presence of central venous catheter, and bio incompatible dialyzers are several factors triggering the development of oxidative stress. Oxidative stress generated can lead to plasma depletion of antioxidants such as serum bilirubin. Antioxidant supplementation may take an overall protective role.

CONCLUSION

Bilirubin has been recognized as an important endogenous antioxidant and anti-inflammatory molecule thereby reducing cardiovascular mortality, which remains a major cause of death in haemodialysis patients. The increase in serum bilirubin is due to haemoconcentration. Higher serum bilirubin levels even within normal range is said to reduce all-cause mortality.

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