Research Article

Evaluation of Malondialdehyde, Vitamin C and Reduced Glutathion Levels in Pre and Post Hémodialysis Patients

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ABSTRACT

Oxidative stress often occurs in chronic hemodialysis. The aim of the present study was to evaluate the level of vitamin C, reduced glutathione and malondialdehyde products of lipid peroxidation in a group of patients on haemodialysis at the Douala General Hospital. Twenty-six patients (13 males and 13 females) on dialysis twice per week and twenty-six healthy subjects corresponding to the control group (13 males and 13 females) were recruited. The results showed significantly higher level (p<0.05) of malondialdéhyde (MDA) after haemodialysis compared to a control group. In addition, the vitamin C levels decrease significantly after haemodialysis (p < 0.05). The level of reduced glutathione before haemodialysis was significantly low compared to the control group but increased significantly after haemodialysis. We also found that there was a significant positive correlation (p < 0.05) between MDA and vitamin C after a hemodialysis session.

Keywords: Oxidative stress, Free radicals, antioxidant, haemodialysis, Atherosclerosis.

INTRODUCTION

Hemodialysis (HD) is the most common technique used to treat end-stage renal disease. Methods of dialysis have not only significantly extended the lifespan of patients, but have also enhanced quality life. However, the contact of blood with the dialysis membrane is not inert; it involves interactions with soluble or cellular components of blood, protein adsorption phenomena, adhesion and cell activation leading to the generation of reactive oxygen, responsible for clinical deterioration[1,2]. Oxidative stress in haemodialysis seems to be greatly involved in the complications observed at long-term in subjects undergoing dialysis. Among these complications, four dominate their severity and draw backs as a result which should be prevented or delayed: the amylose β2-microglobulin, malnutrition, anaemia and the development of atherosclerosis aggravated by calcium deposits. Cardiovascular diseases constitute the major cause of morbidity and mortality in this population[3,5].

The study of Ferretti[15] and his collaborators in 2008 shows, however, that the administration of vitamin C alone in patients under haemodialysis increases the ascorbic acid level in serum with potentially beneficial effects on lipid and malondialdehyde levels and on the characteristics of the plasma polypeptide. Similarly, intravenous administration of vitamin C in patients with renal failure has a beneficial effect on the control of anaemia. In Western countries, monitoring oxidative stress in haemodialysis patients appears to be adequately controlled which is not yet the case in Africa general and Cameroon in particular where few studies have so far

been conducted on oxidative stress and antioxidant status in patients on haemodialysis. Increased oxidative stress remains today a major public problem and may increase morbidity and mortality in patients on Haemodialysis in Cameroon.

In a bid to contribution in improving the therapeutic management of patients on haemodialysis in Cameroon, we seek to evaluate in this study, a marker of lipid peroxidation and the level of vitamin C and reduced glutathione in plasma in a group of patients on haemodialysis at the Douala General Hospital and to compare these levels to that of healthy persons.

MATERIALS AND METHODS

The study participants were 26 patients (13 men and 13 women) aged between 20 and 78 years with end-stage renal disease (ESRD) who were placed on dialysis two (02) times per week for 4 hours with the use of Polysulfone membranes at the haemodialysis centre within the Douala General Hospital. Also, a control group of healthy volunteers made up of 26 healthy people (13 men and 13 women) aged between 28 and 61 years were also recruited. None of the patients was a smoker, nor an alcoholic, nor was suffering from HIV/AIDS. A research authorization No. 116 AR/MINSANTE/HGD/DM/06/13 issued by the Medical Service of the Douala General Hospitalwas obtained for the study. Ethical clearance No. 2013/11/377/L/CNERSH/SP was also issued by the National Ethics Committee on Research on Human Health and a duly signed consent form from each patient who participated in the study was also obtained before inclusion in the study.

Sample collection

Blood samples were collected in heparinized tubes at the end of the fistula needle before and after dialysis. They were then transported wrapped in aluminium foil in a cooler with ice blocks to the Institute of Medical Research and Study of Medicinal Plants (IMPM). In healthy patients, each sample was collected when they had not eaten at the level of the anterior region of the forearm with a tourniquet and needle vacutainer. After centrifugation of the blood at 3000 rpm for 10 minutes, the plasma was decanted and transferred to eppendorfs and then frozen at -20 ° C. Creatinine, MDA, vitamin C and reduced glutathione were measured throughout by spectrophotometric methods.

Determination of plasma creatinine

Creatinine was determined by the method of Bartels et al.[4] In a test tube, 1 ml of the working solution was pipetted. 0.1 ml of sample or standard was added and the whole is mixed quickly. The increase in optical density (520 nm) after one minute was read 20 seconds after the stabilization of the mixture.

Determination of Malondialdehyde (MDA)

The dosage of MDA was done by the method of TBARS (Thiobarbituric Acid Reactive Substances). 100 μl of sample and 400 μl of TBA reagent were pipetted into glass test tubes and tightly closed. The mixture was heated in a water bath at 100 ° C for 15 minutes then cooled in a cold water bath for 30 minutes with the tubes open to permit the escape of gases formed during the reaction. Centrifuge at 3400 revolutions/minute for 5 minutes and read the absorbance of the supernatant at 532 nm.

Estimation of the level of vitamin C in Plasma

The concentration of vitamin C (ascorbic acid) was determined by the spectrophotometric method of Omayer et al.,[6]. A solution of 1.5 ml of 6 % TCA was added to 0.5 ml of plasma and centrifuged at 3500 rpm for 2 min. The supernatant was removed and mixed with 0.5 ml of reagent 2,4- dinitrophenylhydrazine (2 g of DNPH and 4 g of thio-urea in 100 ml of 9N sulfuric acid) and incubated at room temperature for 3 hours. After incubation, 2.5 ml of sulfuric acid cooled in ice was added into the tubes and the resultingcolor was read at 530 nm after 30 minutes.

Determination of reduced glutathione

The concentration of reduced glutathione was determined by the Ellman method[7]. 20 ml f plasma and 3 ml of Ellman reagent were pipetted and mixed. The mixture was incubated for 60 minutes and the optical density was read at 412 nm against the white (plasma replaced by phosphate buffer).

Statistical analysis of results

The data obtained was analyzed with Microsoft Excel software. The statistical analysis was done using the Statistical Package for the Social Sciences (SPSS Version 20.0) software. The results were expressed as mean ± standard difference. The comparisons between the mean values before and after dialysis and the control group was done using the Wilcoxon non-parametric test appropriate for small sample and taking into account the differences in levels within the pairs . Correlations between various markers were performed using the Spearman correlation matrix. The threshold probability (p-value) $p < 0.05$ was chosen as level of significance.

RESULTS

General characteristics of the study population

To see if there was consistency in the two population groups used, the mean age, weight, height and body mass index (BMI) were calculated in patients on haemodialysis on the one hand and the patients in the control group on the other hand (Table 1). Moreover, the average duration of haemodialysis in the group of patients on haemodialysis was also determined.

Table 1: General characteristics of the study population

Variable	Age in Years (μ ± δ)	Weight in Kg (μ ± δ)	Height in meters (μ ± δ)	BMI in Kg/m^2 (μ ± δ)	Duration of haemodialysis in months
Patients on haemodialysis n = 26	53,42 ± 3,06	68,01 ± 3,12	1,67 ± 0,01	21,53 ± 1,17	37,77 ± 6,86
Control Group n=26	43,61 ± 2,19	71,84 ± 3,31	1,65 ± 0,015	25,32 ± 0,96	-

BMI: Body Mass Index, μ: Mean, δ: Standard Difference

Table 2: Comparison of plasma creatinine in haemodialysis patients (before and after dialysis) and the control group.

	Befor hémodialysis	After hémodialysis	Control group
créatinine	74,03[b]	9,33[a]	7,47[b]

NB: Values bearing different letters are statistically different (p < 0.05)

The age, height and weight were not significantly different in the two groups. We noted no overweight in patients on haemodialysis. Contrarily, the subjects in the control group were overweight.

Comparison of plasma creatinine in patients on haemodialysis and the control group

To see the effectiveness of treatment of the polysulfone membrane on metabolic waste   (Table 2), creatinine levels was evaluated in haemodialysis patients before and after dialysis and in the control group.

We observed a significantly elevated level of creatinine in plasma in patients on haemodialysis as compared to the control group. This rate drops significantly after dialysis (p < 0.05). This signifies that the treatment of patients by haemodialysis method drastically decreases the rate of plasma creatinine.

Comparison of malondialdehyde (MDA) in patients on hemodialysis (before and after) and the control group

The plasma MDA was assessed in patients on haemodialysis before and after dialysis and in the control group. Figure 1 below shows the results obtained
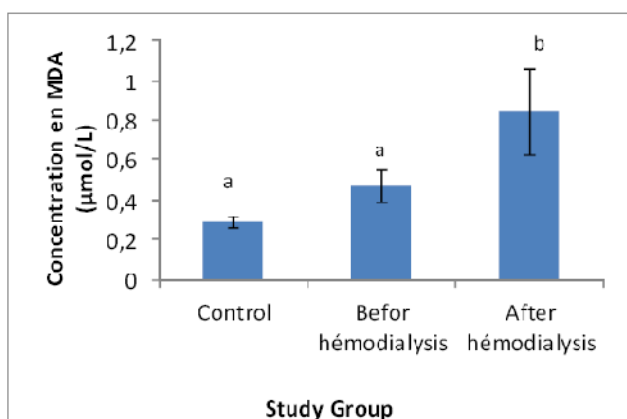
NB: Values bearing different letters are statistically different (p < 0.05)

Figure 1: Comparison of plasma MDA in hemodialysis patients (before and after dialysis) and the control group

No significant difference was observed between the control group and hemodialysis patients before dialysis. However, there was a significant difference between patients on haemodialysis after dialysis and the control group (p < 0.05). This shows that haemodialysisisfavours the production of free radicals.

Comparison of plasma levels of vitamin C in hemodialysis patients (before and after dialysis) and the control group

The level of vitamin C in plasma was evaluated in patients on haemodialysis patients before and after dialysis and in the control group. Figure 2 below shows the results obtained.

No significant difference was observed between the control group and haemodialysis patients before dialysis. However, there was a significant difference between haemodialysis patients after dialysis and the control

group (p < 0.05). This demonstrates that vitamin C is dialyzed during haemodialysis and its level tends to decrease.
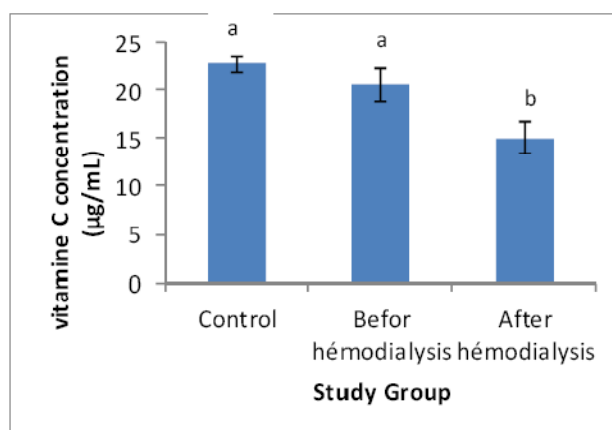
NB: Values bearing different letters are statistically different (p < 0.05)

Figure 2: Comparison of plasma levels of vitamin C in hemodialysis patients (before and after dialysis) and the control group

Classification of patients according to their plasma concentrations of vitamin C both before and after dialysis

To see the number of patients deficient in vitamin C after a haemodialysis session, a classification of patients according to their vitamin C in plasma was performed. Figure 3 below shows the results obtained
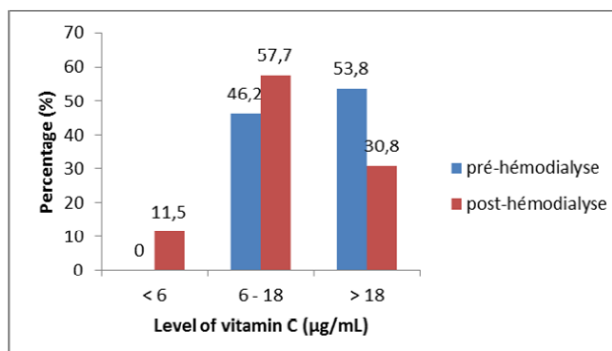

Figure 3: Classification of patients according to their plasma concentrations of vitamin C both before and after dialysis.

We observed before a haemodialysis session, that 46.2% of patients had a concentration of vitamin C in normal against 53.8% of patients above the normal. After haemodialysis, 30.8% of patients were maintained above the normal levels of vitamin C, 57.7% in the normal against 11.5% of patients in deficiency of vitamin C.

Comparison between reduced Glutathione in patients on haemodialysis (before and after dialysis) and control group

GSH levels were evaluated in haemodialysis patients before and after dialysis and in the control group. Figure 4 below shows the results obtained.
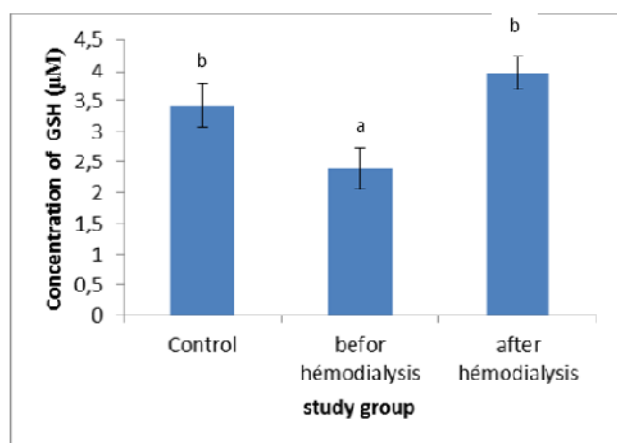
NB: Values bearing different letters are statistically different (p < 0.05)

Figure 4: Comparison of reduced glutathione between hemodialysis patients (before and after dialysis) and the control group

The level of reduced glutathione was significantly higher in patients in the control group compared to that of patients on haemodialysis before dialysis. However, this level increased significantly after dialysis (p < 0.05). This means that reduced glutathione is dialyzed during haemodialysis.

Correlation between the various parameters before and after the dialysis in the general population

A correlation between the different parameters (MDA, GSH and vitamin C) was performed in patients on haemodialysis before and after dialysis. Table 3 below shows the results obtained.

There was a significant positive correlation (p < 0.05) between MDA and vitamin C after a haemodialysis session.

Table 3: Correlation matrix of different parameters before and after dialysis

Parameters	MDA Before	MDA After	Vit C Before	Vit C After	GSH Before	GSH After
MDA before	1.000	0.138	0.180	0.063	-0.050	-0.267
(p-value)	.	0.502	0.380	0.760	0.810	0.187
MDA after	0.138	1.000	0.100	**0.417***	-0.276	-0.301
(p-value)	0.502	.	0.628	**0.034**	0.172	0.135
vit C before	0.180	0.100	1.000	0.486*	-0.229	-0.186
(p-value)	0.380	0.628	.	0.012	0.261	0.364
vit C after	0.063	**0.417***	0.486*	1.000	-0.284	-0.187
(p-value)	0.760	**0.034**	0.012	.	0.159	0.360
GSH before	-0.050	-0.276	-0.229	-0.284	1.000	0.377
(p-value)	0.810	0.172	0.261	0.159	.	0.057
GSH after	-0.267	-0.301	-0.186	-0.187	0.377	1.000
(p-value)	0.187	0.135	0.364	0.360	0.057	.

*the correlation is significant with p < 0.05

DISCUSSION

It appears from this study that the treatment of haemodialysis patients in polysulfone membrane significantly lowers creatinine levels (p < 0.05), thereby significantly improving the clinical condition of the patient. Our results confirmed those of Batta[8]; Eiselt et al.,(1996) who after studying the effect of different dialysis membranes on various biological parameters, reported that the use of polysulfone membrane in the treatment of patients on haemodialysis significantly lowered the parameters, reflecting the purifying capacity of the membrane with significant reduction of creatinine levels.

The significant increase (p < 0.05) of malondialdeyde after dialysis in this study demonstrates the oxidation of the lipid cell membrane by free radicals. Although there are controversial results in the literature indicating that

haemodialysis sessions could improve the lipid profile in patients with a prior pro-oxidant state such as the state of uremia[10], our results confirmed those of other studies that have suggested an increase in lipid peroxidation in haemodialysis[11-13]. Moreover, some studies reported no significant difference in plasma levels of malondialdehyde after dialysis and the control group[9].

The work of Morena et al.[14] on the French population shows that the treatment by haemodialysis is the main source of increased oxidative damages in patients undergoing haemodialysis instead of the disease itself. The increase of the level of malondialdehyde in plasma after a haemodialysis session in this study signified that the technique of haemodialysis is probably the main source of free radicals and increased oxidative stress with a drop in certain antioxidants like vitamin C.

Many studies have shown a variation of the level of vitamin C before and after haemodialysis. In this study, the level of viamin C was significantly low after a haemodialysis session relative to the control group (p< 0.05). In the male patients undergoing haemodialysis, the level of vitamin C was significantly low before and after dialysis as compared to the control group. This has been demonstrated by Morena *et al.,*[14]. Ferreti *et al.*[15] showed in another studies that vitamin C is effectively dialyzed and as such, its level tend to be low in the absent of supplements. In female patients undergoing haemodialysis, the level of vitamin C drops significantly after each dialysis session; and remains lower as compared to the control group. Moreover, this level is higher but not significant in patients before dialysis as compared to the control group. Epidemiological studies notably, SU-VI-MAX[16] assigned the level of vitamin C necessary to fight against the production of free radicals at 6μg/ml. In this study, after a haemodialysis session, 30.8% of the patients had their vitamin C levels above the normally acceptable level with 57.7% in the normal against 11.5% in the patients (all male) with vitamin C deficiency. This deficiency could have been implicated in the long term complications like anaemia, malnutrition, atherogenesis, observed in patients undergoing haemodialysis. Glutathione is the main hydro-soluble antioxidant in the cytosol and directly participate in the destruction of oxygenated reactive compounds. In this study, the level of reduced glutathione seems to be significantly lower (p<0.05) in patients before haemodialysis with respect to the control group. Our results are similar to those of previous studies[17,18]. Meanwhile, in the study carried out by Durak *et al.,*[19] the level of GSH was not significantly different as compared to the control group before dialysis. Moreover, due to the antioxidant property of GSH, it plays a key role in the antioxidant network by recycling oxidized forms of vitamin C, restoring its antioxidant power. The significant increase (p<0,05) in the level of GSH after a haemodialysis session in patients could explain the significant reduction in the level of vitamin C in plasma followed by a significant increase in MDA after haemodialysis. This increase can be costly to the cell in the case where the cells' reserve in glutathione is seen to be low. In the general population of patients undergoing haemodialysis, we observed a significant (p<0.05) positive correlation (r = 0.417) between MDA and vitamin C after dialysis.

CONCLUSION

We can then conclude that, a single haemodialysis session even when carried out with a biocompatible membrane, seems to play an important role in the disequilibrium between the production of FRO and the antioxidant defence. Consequently, it is appropriate to consider that the increase of stress oxidants is an important therapeutic target in patients undergoing haemodialysis at the Douala General Hospital and susceptible in increasing morbidity and mortality within this population.

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