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Evaluation of Non-Enzymatic Antioxidant Levels in Men and Women in Pre and Post Hemodialysis

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ABSTRACT

Oxidative stress often occurs in men and women 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 men and women on hemodialysis 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 levels ($p<0.05$) of malondialdehyde (MDA) after hemodialysis compared to the control in the women group. In addition, the vitamin C levels decrease significantly after hemodialysis ($p <0.05$) compared to the control in the men group. The level of reduced glutathione before hemodialysis was significantly low compared to the control group but increased significantly after hemodialysis. The results of this study have shown that there is considerable oxidative stress in patients with chronic renal failure which is further exacerbated by hemodialysis.

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 and adhesion and cell activation leading to the generation of reactive oxygen species, responsible for clinical deterioration [1, 2]. Oxidative stress in hemodialysis 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, anemia 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 hemodialysis 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 anemia.

In a bid to contribute in improving the therapeutic management of patients on hemodialysis 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 men and women on hemodialysis 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 hemodialysis center 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 Hospital

was 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.

i) 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 aluminum 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 eppendorf tubes and then frozen at -20°C. Creatinine, MDA, vitamin C and reduced glutathione were measured throughout by spectrophotometric methods.

ii) 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 was mixed quickly. The increase in optical density (520 nm) after one minute was read 20 seconds after the stabilization of the mixture.

iii) Determination of Malondialdehyde (MDA)

The measurement 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.

iv) 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 resulting color was read at 530 nm after 30 minutes.

v) 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).

vi) 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

1) Comparison of plasma Creatinine in patients on hemodialysis and the control group in function of gender.

To see the effectiveness of treatment of the polysulfone membrane on metabolic waste (Table 1**)** Creatinine levels was evaluated in hemodialysis men and women before and after dialysis and in the control group.

Table 1: Comparison of plasma creatinine in hemodialysis patients (before and after dialysis) and the control group.

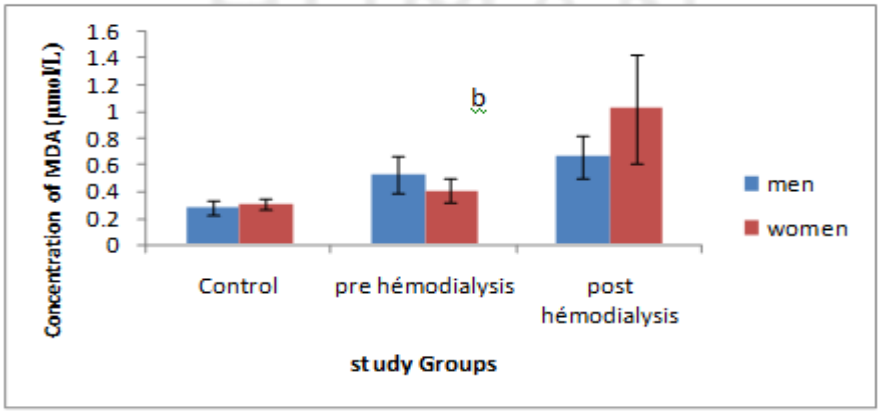
	Creatinine in mg/l		
	Before hemodialysis	After hemodialysis	Control group
men	$81,13 \pm 5,60^{a}$	$10,88 \pm 1,93^{b}$	$7,24 \pm 0,46^{b}$
women	$66,92 \pm 4,21^{a}$	$7,79 \pm 0,69^{b}$	$7,71 \pm 0,67^{b}$

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

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

2) Comparison of MDA in hemodialysis patients (before and after) and the control group in function of gender

The plasma MDA was assessed in patients on hemodialysis before and after dialysis and in the control group in function of gender. This was to enable the visualization of the changes in plasma MDA which occurred during a hemodialysis session in men as well as in women on hemodialysis. Figure 1 below shows the results obtained.



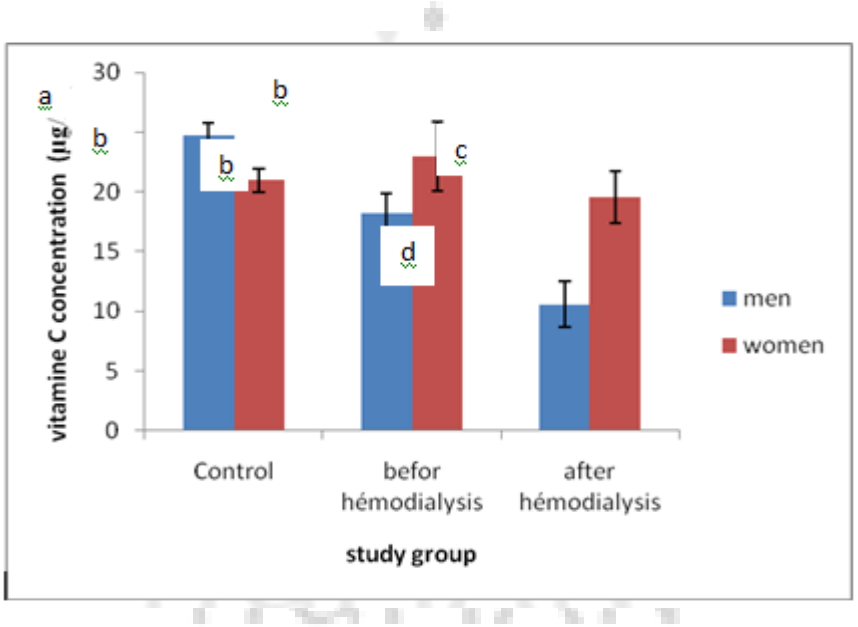
NB: Values bearing different letters are statistically different ($p < 0.05$)

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

No significant difference was observed between the control group and hemodialysis patients before dialysis in both sexes. However, there was a significant increase in MDA in female patients on hemodialysis compared to their male counterpart. This means that female patients are more susceptible to increased rate of lipid peroxidation during a hemodialysis session compared to male.

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

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



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 in terms of gender

The level of vitamin C was significantly higher in male patients in the control group compared to hemodialysis patients of the same sex before dialysis. Moreover, the rate dropped significantly after dialysis ($p < 0.05$). In female patients on hemodialysis, no significant difference was observed compared with patients of the same sex in the control group before dialysis. However, this rate dropped significantly after dialysis.

4) *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 hemodialysis session, a classification of patients according to their vitamin C in plasma was performed. Figure 3 below shows the results obtained.

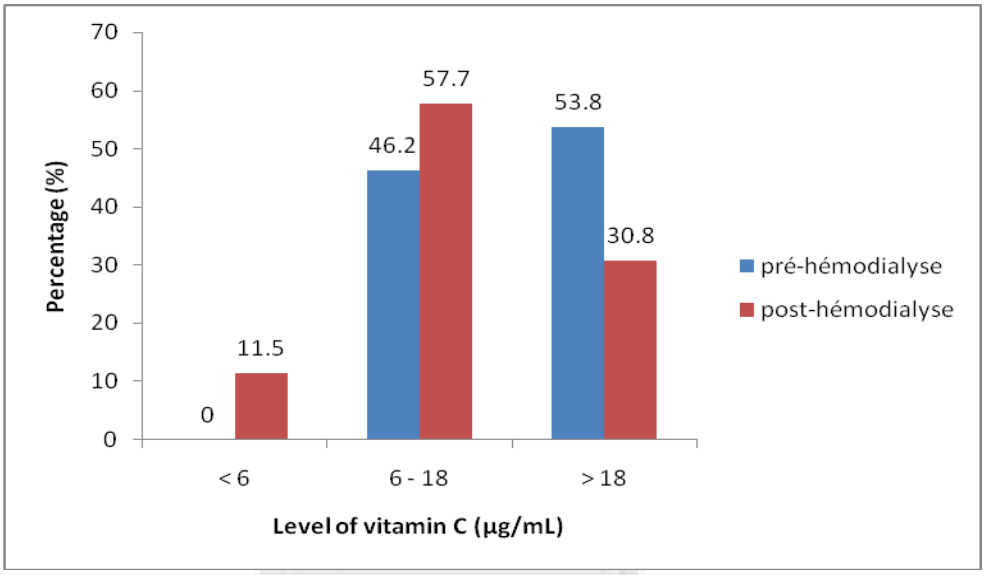


Figure 3: Classification of patients according to their plasma concentrations of vitamin C both before and after dialysis.

We observed before a hemodialysis session, that 46.2% of patients had a concentration of vitamin C in normal against 53.8% of patients above the normal. After hemodialysis, 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.

5. Comparison of reduced glutathione between hemodialysis patients (before and after dialysis) and the control group in terms of gender.

GSH levels were evaluated in hemodialysis patients before and after dialysis and in the control group in terms of gender. Figure 4 below shows the results obtained.

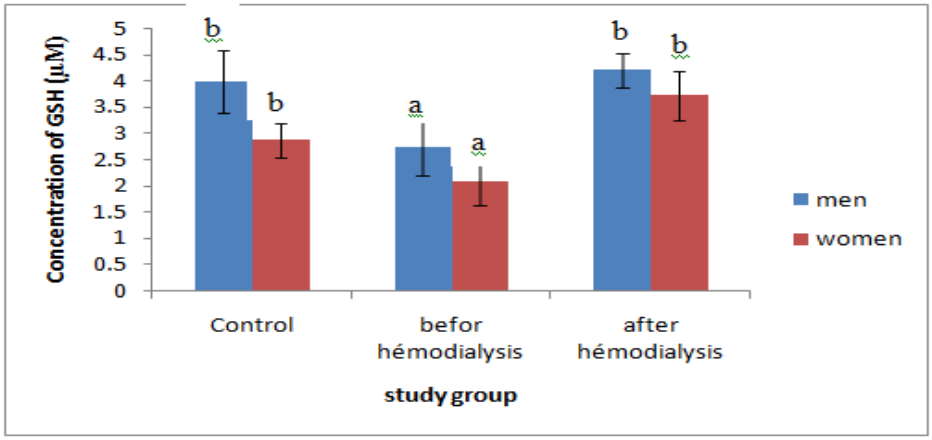


Figure 4: Comparison of reduced glutathione between hemodialysis patients (before and after dialysis) and the control group in terms of gender

The level of reduced glutathione was significantly higher in patients in the control group of both sexes compared with patients of the same sex in hemodialysis. However, this rate increased significantly after dialysis ($p < 0.05$) in both sexes.

6. Correlation between the various parameters before and after the dialysis in male patients

A correlation between the different parameters (MDA, vitamin C and GSH) was conducted in male patients undergoing hemodialysis before and after dialysis. Table 2 below shows the results obtained.

Table 2: Correlation matrix of parameters before and after dialysis in male patients

Parameters	MDA before	MDA after	Vit C before	Vit C after	GSH before	GSH after
MDA before	1.000	0.038	**0.641***	0.182	-0.069	-0.130
p-value	.	0.901	**0.018**	0.553	0.823	0.673
MDA after	0.038	1.000	0.209	**0.735****	-0.485	-0.229
p-value	0.901	.	0.493	**0.004**	0.093	0.452
vit C before	**0.641***	0.209	1.000	0.514	-0.109	-0.315
p-value	**0.018**	0.493	.	0.072	0.723	0.295
vit C after	0.182	**0.735****	0.514	1.000	-0.342	**-0.576***
p-value	0.553	**0.004**	0.072	.	0.253	**0.039**
GSH before	-0.069	-0.485	-0.109	-0.342	1.000	0.680*
p-value	0.823	0.093	0.723	0.253	.	0.010
GSH after	-0.130	-0.229	-0.315	**-0.576***	0.680*	1.000
p-value	0.673	0.452	0.295	**0.039**	0.010	.

** The correlation is significant at $p < 0.01$; *the correlation is significant at $p < 0.05$

There was a significant positive correlation ($p < 0.05$) between MDA and vitamin C before dialysis and after dialysis ($P < 0.01$). A significant negative correlation ($p < 0.05$) between reduced glutathione and vitamin C was observed after dialysis.

7. Correlation between the various parameters before and after the dialysis in female patients

A correlation between the different parameters (MDA, vitamin C and GSH) was conducted in female patients undergoing hemodialysis before and after dialysis. Table 3 below shows the results obtained.

Table 3: Correlation matrix of parameters before and after dialysis in female patients

Parameters	MDA before	MDA after	Vit C before	Vit C after	GSH before	GSH after
MDA before	1.000	0.253	-0.093	-0.022	-0.050	-0.313
p-value	.	0.405	0.762	0.943	0.871	0.297
MDA after	0.253	1.000	-0.082	0.137	0.017	-0.242
p-value	0.405	.	0.789	0.655	0.957	0.426
vit C before	-0.093	-0.082	1.000	0.412	-0.236	-0.247
p-value	0.762	0.789	.	0.162	0.438	0.415
vit C after	-0.022	0.137	0.412	1.000	0.100	0.280
p-value	0.943	0.655	0.162	.	0.745	0.354
GSH before	-0.050	0.017	-0.236	0.100	1.000	0.269
p-value	0.871	0.957	0.438	0.745	.	0.374
GSH after	-0.313	-0.242	-0.247	0.280	0.269	1.000
p-value	0.297	0.426	0.415	0.354	0.374	.

There is no correlation between the various parameters before and after dialysis in female patients undergoing hemodialysis.

DISCUSSION

It appears from this study that the treatment of hemodialysis 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 hemodialysis 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 hemodialysis 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 hemodialysis[11,12,13]. 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 showed that the treatment by hemodialysis is the main source of increased oxidative damages in patients undergoing hemodialysis instead of the disease itself. The increase in the level of malondialdehyde in plasma after a hemodialysis session in this study signified that the technique of hemodialysis 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 hemodialysis. In this study, the level of vitamin C was significantly low after a hemodialysis session relative to the control group ($p< 0.05$). In the male patients undergoing hemodialysis, 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 study that vitamin C is effectively dialyzed and as such, its level tend to be low in the absent of supplements. In female patients undergoing hemodialysis, 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 hemodialysis 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 hemodialysis. 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 hemodialysis 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 hemodialysis session in patients could explain the significant reduction in the level of vitamin C in plasma followed by a significant increase in MDA after hemodialysis. This increase can be costly to the cell in the case where the cells' reserve in glutathione is seen to be low. In the men, a significant ($p<0.05$) negative correlation($r= -0.576$) between GSH and vitamin C followed by a significant ($p<0.01$) positive correlation ($r = 0.735$) between MDA and vitamin C was observed. This can be justified by the fact that when vitamin C levels reduce, the reduced glutathione increases to either increase the antioxidant power of vitamin C or to fight against the increase in oxidative stress.

CONCLUSION

We can then conclude that, a single hemodialysis 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 defense in men and women hemodialysis. Consequently, it is appropriate to consider that the increase in oxidative stress is an important therapeutic target in patients undergoing hemodialysis at the Douala General Hospital and susceptible in increasing morbidity and mortality within this population.

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