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Hemodialysis-associated Oxidative Stress: Comparison of Helixone® and Polysulfone® Dialyzer

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Authors' contributions

This work was carried out in collaboration among all authors. Author ME designed the study, wrote the protocol and the manuscript and did plasma oxidative stress analysis and statistical data analysis. Author FZB managed the patients, did plasma oxidative stress analysis and collected all data. Authors KAS, CM and MA managed the patients. Author TSH designed the study and the protocol managed the patients and revised the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: Patients at end stage renal failure require hemodialysis, a process that contributes to oxidative stress, where the quality of hemodialysis membrane plays a key role.
Aim of the Study: We sought to investigate the effect of two different hemodialysis membranes (Helixone® vs Polysulfone®) on several blood biologic and oxidative stress parameters.
Patients and Methods: Among 86 patients of our hemodialysis facility at the University Hospital, 12 patients were included in the study according to inclusion and exclusion criteria. Patients used Polysulfone® membrane in a first step of the study, then they switched to Helixone® membrane.

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Under the two kinds of membranes, we measured routine biologic parameters, and pre- and post-dialysis plasma oxidative stress parameters (malondialdehyde and Total Anti-Oxidant Activity, as assessed respectively, by the method of thiobarbituric acid, and an anti-oxidant activity commercial kit).

Results: With both Polysulfone® and Helixone® dialyzers, there was an important and significant increase of malondialdehyde upon a single hemodialysis session, however, the increase of malondialdehyde concentration was significantly reduced with Helixone® dialyzer as compared to Polysulfone® one. Plasma total anti-oxidant activity was reduced significantly upon a single hemodialysis session with both dialyzers, however, such a reduction was significantly higher with Polysulfone® (28% reduction) than with Helixone® (11.7% reduction) dialyzer. The levels of other routine blood parameters related to the performance of dialysis process (urea, uric acid, creatinine) under both dialyzers were similar.

Conclusion: Hemodialysis-associated oxidative stress, as assessed by MDA concentration and plasma total anti-oxidant activity, seems to be significantly reduced with Helixone® dialyzer when compared to Polysulfone® dialyzer. This difference may bring positive impact on hemodialysis-associated side effects on cardiovascular and cerebrovascular systems.

Keywords: Hemodialysis; membrane dialyzer; oxidative stress; malondialdehyde.

1. INTRODUCTION

In the last two decades, hemodialysis process was ameliorated thanks to novel brands of membrane dialyzers known as biocompatible, such as synthetic and cellulose modified dialyzers [1-3]. Biocompatible dialyzers are known to induce less leucopenia and complement activation. New brands of synthetic dialyzers were produced, whose filtration qualities were improved by the so-called “Nano Controlled Spinning” technology [4,5]. The new dialyzers have better performance and are safer, because they have a better number of pores with a better diameter and a better anatomical characteristics. Hemodialysis by itself is a pro-inflammatory and pro-oxidant process [6-12], and the goal in developing new brands of dialyzers is also to reduce inflammation and oxidative stress. An example of that is vitamin E-coated membranes [13,14]. Despite the above progress, the process of hemodialysis is still associated with high cardiovascular mortality and morbidity [15,16]. Other risk factors such as dyslipidemia, hyperglycemia and hypertension are added to the general inflammatory state and malnutrition in hemodialysis patients [17,18]. Hemodialysis process is known to induce an oxidative stress [19-24], which is defined as a decrease of anti-oxidant capacity of the organism (less anti-oxidant enzymes, less anti-oxidant molecules], and a subsequent production of oxygen free radicals that oxidize molecular constituents of the cells, such as proteins, lipids and nucleic acids [25,26]. Malondialdehyde (MDA) is a toxic product derived from fatty acid peroxidation [27-29]. It is generally accepted that MDA represents a good biomarker of oxidative stress in physiopathology. It can be measured in biologic liquids by the method of thiobarbituric acid reactive substances (TBARS) with or without high pressure liquid chromatographic analysis [30,31]. Although this method is not very specific, it was used in many studies with consistent results about the generation of MDA in different experimental and pathologic situations. In the present study, patients of our hemodialysis facility experienced a replacement of membrane dialyzer, those, switching from Polysulfone® to Helixone® membranes. During this dialyzer replacement, we sought to investigate the effect of quality of dialysis membrane on blood parameters, especially those related to oxidative stress and molecule’s filtration. Here, we demonstrate the improvement of oxidative stress in patients, where hemodialysis-associated MDA production was significantly reduced upon switching from Polysulfone® to Helixone® dialyzer.

2. PATIENTS AND METHODS

2.1 Patients

Among 86 patients of our hemodialysis facility at the University Hospital, 12 patients were enrolled in the study, all of them were males, above 15 years of age. Exclusion criteria were: CRP above 6, infectious episode or hospitalization in the last 15 days preceding the study, diabetic and smoking patients. Serology results of patients were negative to HIV, hepatitis B and C. Patients included in the study did not receive erythropoietin nor copper

therapy during the study. To participate in the study, patients signed a written consent, and the study was approved by a local ethic committee.

2.2 Hemodialysis Session

In a first step, patients were using Polysulfone® (F6HPS) membrane dialyzer, and blood samples were obtained both before and after each hemodialysis session. Plasma or sera were prepared and frozen at – 80℃ until use. Plasma was then used to assess MDA and plasma total anti-oxidant activity. Serum was used to assess other routine blood parameters. In our hemodialysis facility at the hospital, Polysulfone® dialyzers were replaced by Helixon® dialyzers. For this purpose, as a second part of the present study, patients have switched to Helixon® (Fx8) membrane dialyzer, and six weeks later, blood samples were obtained both before and after hemodialysis session to assessing MDA, plasma total anti-oxidant activity and the other routine blood parameters. For hemodialysis sessions, bicarbonate buffer was used and pomp flow rate was set at more than 300 ml /min (Kt/V above 1.2). Sodium heparin was used as anti-coagulant. Both membrane dialyzers were from Fresenius Medical Care.

2.3 MDA Measurement

The method of TBARS [30,31] was used to determine MDA levels in blood plasma of patients. The method is based on the production of an adduct which is produced upon a chemical reaction of MDA and thiobarbituric acid. The above adduct, after extraction, was measured by reading the absorbance of the reaction mixture at 532 nm. Tetramethoxypropane was used as a chemical standard in the assay, as detailed in a previous paper on plasma MDA production in hemodialysis patients [20]. All reagents were of analytical grade, and were purchased from Sigma Aldrich.

2.4 Total Anti-oxidant Activity

Blood plasma was used to determine total anti-oxidant activity, according to a method described in a commercial kit of Antioxidant Status Assay, purchased from Calbiochem. Briefly, the assay is based on the ability of plasma samples to inhibit the oxidation of ABTS (2,2-Azino-di-[3-ethylbenz-thiazoline sulphonate) substrate to $ABTS^+$ product by metmyoglobin. The amount of $ABTS^+$ product is monitored by reading the absorbance of the reaction mixture at 600 nm.

2.5 Blood Parameters

Routine biochemistry measurements of blood samples were performed at the Central Laboratory of Hassan II University Hospital according to standard methods.

2.6 Statistical Analysis

Data are presented as mean ± SD. Excel software for Windows was used for comparison of mean values with paired Student's T-test. For plasma MDA measurement, a regression equation was calculated with Excel software in order to obtain a MDA calibration curve, where a coefficient of determination (R^2) was always above 0.99.

3. RESULTS

Characteristic of patients and the origin of their nephropathy are shown in Table 1.

Table 1. Characteristics of study population

Variable	Value
Sex ratio (M/W)	12/0
Age (years)	31.50±8.0
Duration in hemodialysis therapy (months)	48.58±25
Origin of nephropathy:	
Hypertension	3
Glomerular	2
Genetic	1
Not determined	6

Blood parameters did not differ significantly under both types of membranes (Table 2).

The filtration capacity of both membranes for urea, uric acid, and creatinin were similar, as assessed upon a single hemodialysis session (Table 3).

Oxidative stress was estimated through the levels of plasma MDA and plasma total anti-oxidant activity in patient's blood before and after a single hemodialysis session. Calibration curve of MDA measurement is shown in Fig. 1.

MDA levels increased significantly in every patient's blood upon a single hemodialysis session as shown in Fig. 2, and thus, with both kinds of dialysis membrane. Interestingly, among all patients, patient number 9 has an exceptional very high increase of MDA level linked to the hemodialysis process with both kinds of dialysis

membrane (Fig. 2). For the above reason, MDA value of patient number 9 was not taken into account when calculating mean value of MDA of all patients (Inserted table in Fig. 2).

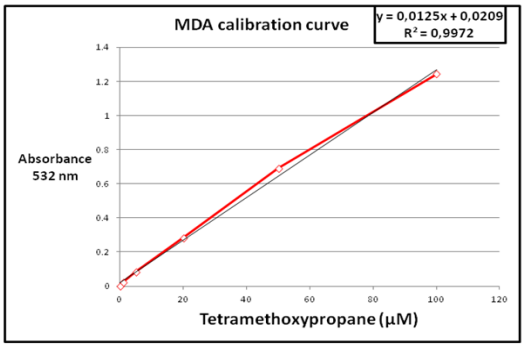


Fig. 1. Calibration curve of plasma MDA analysis

There was more than 375% mean increase of MDA level upon hemodialysis session with Polysulfone® membrane, instead, there was about 189% mean increase of MDA level upon hemodialysis session with Helixone® membrane. The increase of MDA upon a single dialysis session was significantly reduced (33.77% mean reduction) under Helixone® membrane, as compared to Polysulfone® membrane (Fig. 2: 14.58±7.70 vs 9.65±5.19, corresponding to post-dialysis MDA levels on Polysulfone® and Helixone® membranes, respectively. * p = 0.032; refers to comparison between the above means). Total plasma anti-oxidant activity was significantly reduced upon hemodialysis process with both Polysulfone® (25.80% decrease) and Helixon® (11.78% decrease) membranes (Fig. 3). A statistical significant difference (*: p = 0.000) was found between blood total anti-oxidant activity on Polysulfone® and Helixone® membranes upon hemodialysis session (Fig. 3).

4. DISCUSSION

Oxidative stress plays a key role in hemodialysis process [7-12,20-24]. Each single hemodialysis session is a physical burden for end stage renal failure patients, because it needs at least four hours as a median, and it is performed two to three times a week. On the other hand, the contact of blood components and circulating cells with dialyzer membrane leads to cell activation, which is a source of oxygen free radicals that play a crucial role in the inflammatory reaction and molecular damage. Biocompatible dialysis membranes were manufactured in the last two decades in order to reach a convenient and friendly dialysis process, so as to minimize inflammation, leucopenia and complement activation, as well as to improving the quality of blood filtration. In our present study,

Table 2. Blood parameters as measured before hemodialysis under both Polysulfone® and Helixone® membranes

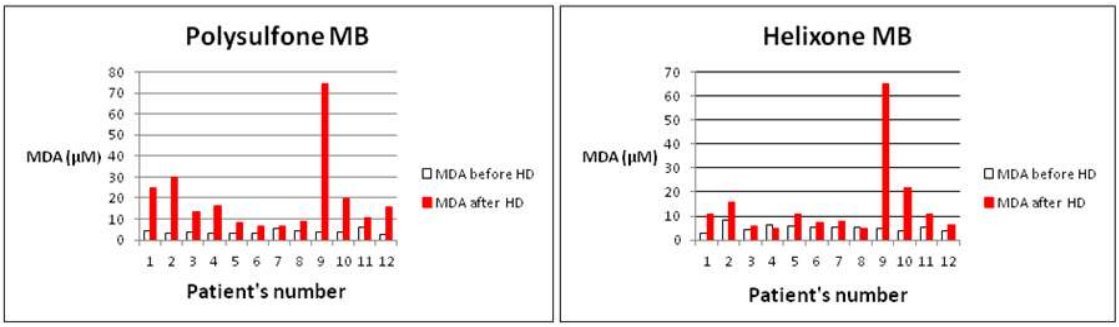
Variable	Polysulfone® membrane	Helixone® membrane	p value
Urea (g/L)	1.17±0.28	1.44±0.26	0.216
Uric acid (g/L)	66.33±5.84	72.33±15.67	0.165
Creatinin (mg/L)	120.25±23.37	123.25±23.97	0.469
Phosphoremia (mg/L)	55.91±12.4	66.25±28.24	0.075
Kalemia (mEq/L)	4.77±0.73	4.90±0.70	0.559
β2 microglobulin (mg/L)	50.82±15.33	57.91±17.08	0.114
Albumine (mg/L)	42.84±1.92	41.58±3.27	0.093
Ferritin (µg/L)	286.33±257.86	353.84±279.31	0.342
Glucose (g/L)	1.07±0.31	1.04±0.18	0.775

Table 3. Filtration properties of Polysulfone® and Helixone® membranes in hemodialyis (HD)

Variable	Polysulfone® membrane			Helixone® membrane		
	Before HD	After HD	p value	Before HD	After HD	p value
Urea (g/L)	1.17±0.28	0.45±0.16	0.000	1.34±0.27	0.34±0.22	0.000
Uric acid (mg/L)	66.33±10.47	15.75±5.84	0.000	72.33±15.67	15.50±12.40	0.000
Creatinin (g/L)	120.25±23.37	48.33±14.24	0.000	123.25±23.97	41.33±21.24	0.000
Phosphoremia (g/L)	55.91±12.4	29.5±8.07	0.000	66.25±28.24	30.66±13.17	0.000
Kaliemia (mEq/L)	4.77±0.73	3.81±0.99	0.000	4.90±0.70	3.54±0.78	0.000

we have shown that switching from Polysulfone® to Helixone® membrane dialyzer brings two positive results on oxidative stress: The first one, is the reduction of MDA production upon hemodialysis, and the second one, is the improvement of plasma total anti-oxidant activity.

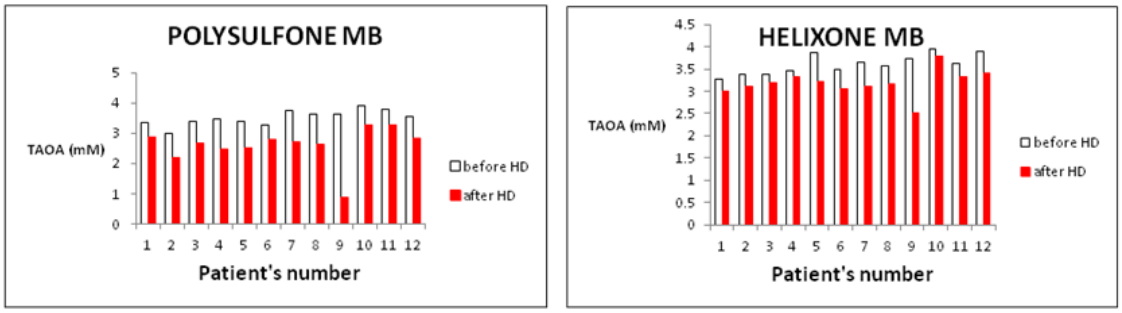
In a previous prospective controlled clinical study [20], we have demonstrated that MDA production was stimulated upon hemodialysis sessions, and that ultrapure dialysis fluid also improved hemodialysis-associated oxidative stress, through reduction of MDA production.



	Polysulfone membrane		Helixone membrane	
Variable	Before HD	After HD	Before HD	After HD
MDA (µM)	3.89 ± 1.02	14.58 ± 7.70 p=0.001	5.10 ± 1.49	9.65 ± 5.19 p=0.019 * p = 0.032

Fig. 2. MDA production before and after hemodialysis (HD) with Polysulfone® and Helixone® membrane dialyzers. Table below shows mean values of MDA concentrations under each membrane dialyzer. p refers to comparison between means before and after HD

**: p = 0.032 refers to comparison between means under each membrane dialyzer*



	Polysulfone membrane		Helixone membrane	
Variable	Before HD	After HD	Before HD	After HD
TAOA (mM)	3.50 ± 0.61	2.59 ± 0.25 p=0.000	3.60 ± 0.22	3.18 ± 0.29 p=0.000 * p= 0.000

Fig. 3. Plasma total anti-oxidant activity (TAOA) before and after hemodialysis (HD) with Polysulfone® and Helixone® membrane dialyzers. Table below shows mean values of plasma TAOA under each membrane dialyzer. p refers to comparison between means before and after HD

**: p = 0.000 refers to comparison between means under each membrane dialyzer*

5. CONCLUSION

Our present data confirm the better biocompatibility of Helixone® dialyzer over Polysulfone® one, and support the idea that more than being a good filter for blood detoxification, Helixone® membranes could play a role in cardiovascular and cerebrovascular protection against hemodialysis-associated oxidative stress.

CONSENT

Patients included in the present study gave their written informed consent in order to participate in the study.

ETHICAL APPROVAL

To perform the present clinical study, the authors obtained the ethical approval from the local ethical committee of the university hospital Hassan II and the Faculty of Medicine and Pharmacy of Fez.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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