

Hemodialysis-associated oxidative stress: Comparison of Helixone® and Polysulfone® dialyzer

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. 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.

30 Key words: Hemodialysis, membrane dialyzer, oxidative stress, malondialdehyde.

31

32 **Introduction**

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

62

63

64 **Patients and Methods:**

65 Patients: Among 86 patients of our hemodialysis facility at the University Hospital, 12
66 patients were enrolled in the study, all of them were males, above 15 years of age. Exclusion
67 criteria were: CRP above 6, infectious episode or hospitalization in the last 15 days preceding
68 the study, diabetic and smoking patients. Serology results of patients were negative to HIV,
69 hepatitis B and C. Patients included in the study did not receive erythropoietin nor copper
70 therapy during the study. To participate in the study, patients signed a written consent, and the
71 study was approved by a local ethic committee.

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

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

91 Total anti-oxidant activity: Blood plasma was used to determine total anti-oxidant activity,
92 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.

Blood parameters: Routine biochemistry measurements of blood samples were performed at the hospital laboratory according to standard methods.

Statistical analysis: Data are presented as mean $\pm$ 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.

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)	31.5 $\pm$ 8.0
Duration in hemodialysis therapy (months)	48.58 $\pm$ 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).

Table 2 : Blood parameters as measured before hemodialysis under both Polysulfone® and Helixone® membrane

Variable	Polysulfone membrane	Helixone membrane
Urea (g/L)	1.17 ± 0.28	1.44 ± 0.26 p= 0.305
Uric acid (mg/L)	66.33 ± 5.84	0.83 ± 0.20 p= 0.113
Creatinin (mg/L)	120.25 ± 23.37	0.32 ± 0.09 p= 0.357
Phosphoremia (g/L)	55.91 ± 12.4	1.45 ± 0.34 p= 0.075
Kaliemia (mEq/L)	4.77 ± 0.73	4.90 ± 0.70 p= 0.559
B2 microglobuline (mg/L)	50.82 ± 15.33	57.91 ± 17.08 p= 0.114
Albumine (mg/L)	42.84 ± 1.92	41.58 ± 3.27 p= 0.093
Ferritine (µg/L)	286.33 ± 257.86	353.84 ± 279.31 p= 0.342
Glucose (g/L)	1.07 ± 0.31	1.04 ± 0.18 p= 0.775

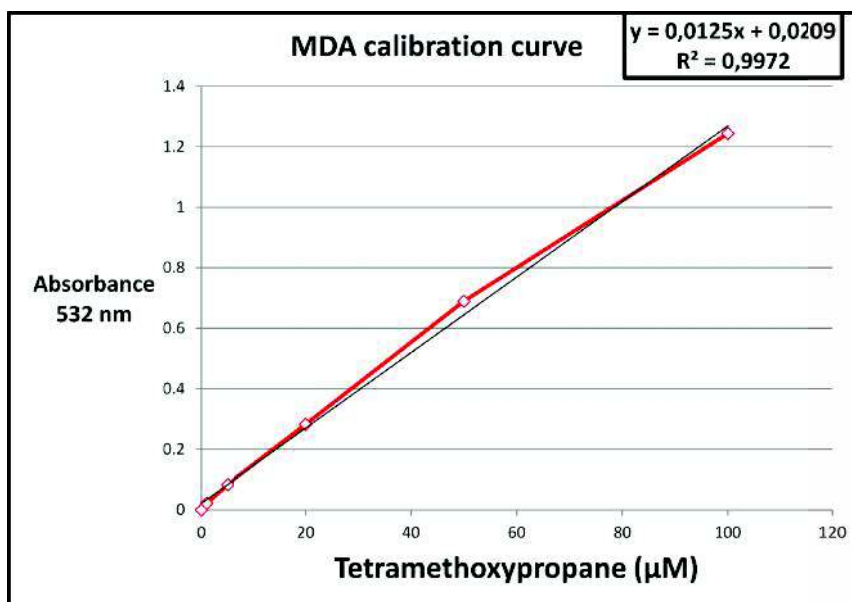
The filtration capacity of both membranes for urea, uric acid, and creatinine were similar, as assessed upon a single hemodialysis session (Table 3).

Table 3: Filtration properties of Polysulfone® and Helixone® membranes in hemodialysis (HD)

	Polysulfone membrane		Helixone membrane	
Variable	Before HD	After HD	Before HD	After HD
Urea (g/L)	1.17 ± 0.28	0.45 ± 0.16 p=0.000	1.34 ± 0.27	0.34 ± 0.22 p= 0.000
Uric acid (mg/L)	66.33 ± 5.84	15.75 ± 10.5 p=0.000	72.33 ± 15.67	15.50 ± 12.40 p=0.000
Creatinine (g/L)	120.25 ± 23.37	48.33 ± 14.24 p=0.000	123.25 ± 23.97	41.33 ± 21.24 p=0.000
Phosphoremia (g/L)	55.91 ± 12.4	29.5 ± 8.07 p=0.000	66.25 ± 28.24	30.66 ± 13.17 p=0.000
Kaliemia (mEq/L)	4.77 ± 0.73	3.81 ± 0.99 p=0.000	4.90 ± 0.70	3.54 ± 0.78 p=0.000

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

Figure 1: Calibration curve of plasma MDA analysis



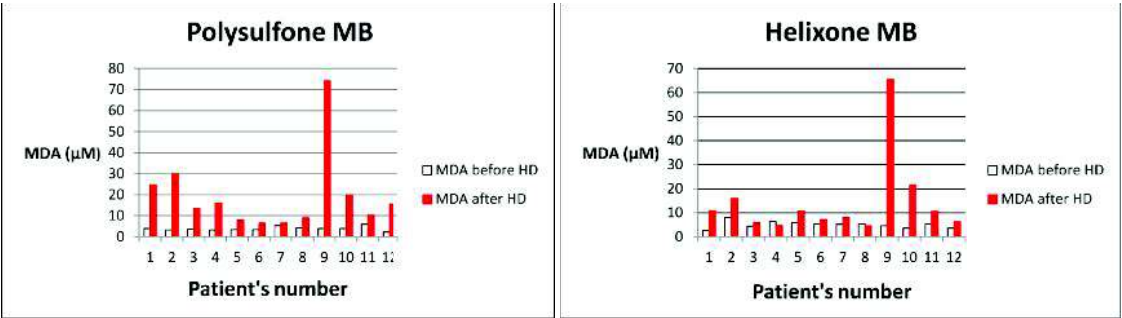
116

117

118

119 MDA levels increased significantly in every patient's blood upon a single hemodialysis
120 session as shown in figure 2, and thus, with both kinds of dialysis membrane. Interestingly,
121 among all patients, patient number 9 has an exceptional very high increase of MDA level
122 linked to the hemodialysis process with both kinds of dialysis membrane (Figure 2). For the
123 above reason, MDA value of patient number 9 was not taken into account when calculating
124 mean value of MDA of all patients (Inserted table in figure 2).

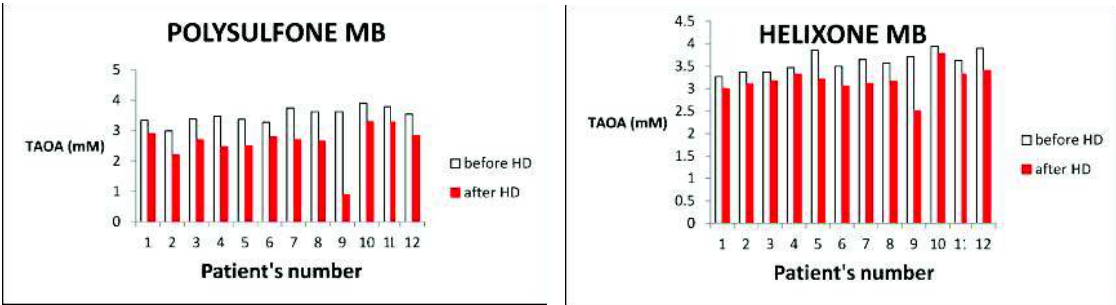
Figure 2: MDA production before and after hemodialysis (HD) with Polysulfone® and Helixone® dialyzers. Table below shows mean values of MDA concentrations under each dialyzer. p refers to comparison between means before and after HD. *: p= 0,032 refers to comparison between means under each membrane.



	Poly;sulfone 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

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 (Figure 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 anti-oxidant activity was significantly reduced upon hemodialysis process with both Polysulfone® (25.80% decrease) and Helixon® (11.78% decrease) membranes (Figure 3). A statistical significant difference (*: p = 0.000) was found between blood total anti-oxidant activity on Polysulfone® and Helixone® membranes upon hemodialysis session (Figure 3).

Figure 3: Plasma Total anti-Oxidant activity (TAOA) before and after hemodialysis (HD) with Polysulfone® and Helixone® dialyzers. Table below shows mean values of anti-oxidant activity. p refers to comparison between means before and after HD. *: p= 0,032 refers to comparison between means under each membrane.



	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

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, 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.

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.

References:

- 1-Kerr PG, Huang L. Review: membranes for haemodialysis. Nephrology (Carlton). 2010, 15(4):381-5.
- 2- Ronco C, Nissenson AR. Does Nanotechnology Apply to Dialysis? Blood Purif 2001; 19: 347–352
- 3- Ronco C, Bowry SK, AlBrendolan A, Crepaldi C, Soffiati G, Fortunato A, Bordoni V, Granziero A, Torsello G, La Greca G. Hemodialyzer: From macro-design to membrane nanostructure; the case of the FX-class of hemodialyzers. Kidney International. 2002 61, 126-142.
- 4- Clark WR, Hamburger RJ, Lysaght MJ. Effect of membrane composition and structure on solute removal and biocompatibility in hemodialysis. Kidney Int. 1999, 56 (6): 2005-15.
- 5- Woffindin C, Hoenich NA. Blood-membrane interactions during haemodialysis with cellulose and synthetic membranes. Biomaterials. 1988, 9 (1): 53-57.
- 6-Samouilidou E, Grapsa E, Karpouza A, Lagouranis A. Reactive oxygen metabolites: a link between oxidative stress and inflammation in patients on hemodialysis. Blood Purif. 2007;25(2):175-8.
- 7-Elshamaa MF, Sabry S, Nabih M, Elghoroury EA, El-Saaid GS, Ismail AA. Oxidative stress markers and C-reactive protein in pediatric patients on hemodialysis. Ann Nutr Metab. 2009; 55 (4): 309-316.

181 8- Tetta C, Biasioli S, Schiavon R, Inguaggiato P, David S, Panichi V, Wratten ML. An
182 overview of haemodialysis and oxidant stress. *Blood Purif.* 1999, 17 (2-3): 118-126.

183 9- Filiopoulos V, Hadjiyannakos D, Takouli L, Metaxaki P, Sideris V, Vlassopoulos D
184 Inflammation and oxidative stress in end-stage renal disease patients treated with
185 hemodialysis or peritoneal dialysis. *Int J Artif Organs.* 2009, 32 (12): 872-882.

186 10- Marques de Mattos A, Marino LV, Ovidio PP, Jordão AA, Almeida CC, Chiarello PG
187 Protein oxidative stress and dyslipidemia in dialysis patients. *Ther Apher Dial.* 2012, 16 (1):
188 68-74.

189 11- Wu CC, Chen JS, Wu WM, Liao TN, Chu P, Lin SH, Chuang CH, Lin YF.
190 Myeloperoxidase serves as a marker of oxidative stress during single haemodialysis session
191 using two different biocompatible dialysis membranes. *Nephrol Dial Transplant.* 2005, 20 (6):
192 1134-1139.

193 12- Mimić-Oka J, Simić T, Djukanović L, Reljić Z, Davicević Z Alteration in plasma
194 antioxidant capacity in various degrees of chronic renal failure. *Clin Nephrol.* 1999, 51 (4):
195 233-241.

196 13- Omata M, Higuchi C, Demura R, Sanaka T, Nihei H. Reduction of neutrophil activation
197 by Vitamin E-coated dialyzer reduces oxidative stress in hemodialysis patients. *Nephron.*
198 2000, 85 (3) : 221-231.

199 14- Clermont G, Lecour S, Cabanne JF, Motte G, Guillard JC, Chevet D, Rochette L Vitamin
200 E modified dialyzer membranes. *Free Radic Biol Med.* 2001, 15; 31 (2): 233-241.

201 15- Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the
202 risks of death, cardiovascular events, and hospitalization. *N Engl J Med.* 2004, 351: 1296–
203 1305.

204 16- Collins, AJ.. Cardiovascular mortality in end-stage renal disease. *Amer J Med. Sci.* 2003,
205 325, 163–167.

206 17- Fernandez-Reyes M. Alvarez F. Sanchez R. Mon C. Iglesias P. Diez J. Inflammation and
207 malnutrition as predictors of patients on hemodialysis. *J Nephrol.* 2002, 15:136-143

208 18- Zimmermann J. Herrlinger S. Pury A. Metzager T. Wanner C. Inflammation enhances
209 cardiovascular risk and mortality in hemodialysis patients. *Kidney Int.* 1999, 55, 648-658.

- 19- Köken T, Serteser M, Kahraman A, Gökçe C, Demir S. Changes in serum markers of oxidative stress with varying periods of haemodialysis. *Nephrology* (Carlton). 2004, 9 (2):77-82.
- 20- Elkabbaj D, Bahadi A, Cherrah Y, Errasfa M, El jaoudi R . Impact of improving quality of dialysis fluid on oxidative stress and lipid profile in hemodialysis patients. *ISRN Nephrol.* 2012 Dec 19; 2013: 717849.
- 21- Stępniewska J, Gołembiewska E, Dołęgowska B, Domański M, Ciechanowski K. Oxidative stress and antioxidative enzyme activities in chronic kidney disease and different types of renal replacement therapy. *Curr Protein Pept Sci.* 2015; 16 (3):243-248
- 22- Clermont G, Lecour S, Lahet J, Siohan P, Vergely C, Chevet D, Rifle G, Rochette L. Alteration in plasma antioxidant capacities in chronic renal failure and hemodialysis patients: a possible explanation for the increased cardiovascular risk in these patients. *Cardiovasc Res.* 2000, 18; 47 (3): 618-623.
- 23- Samouilidou E, Grapsa E. Effect of dialysis on plasma total antioxidant capacity and lipid peroxidation products in patients with end-stage renal failure. *Blood Purif.* 2003; 21 (3): 209-212.
- 24- Jackson P, Loughrey CM, Lightbody JH, McNamee PT, Young IS. Effect of hemodialysis on total antioxidant capacity and serum antioxidants in patients with chronic renal failure. *Clin Chem.* 1995, 41 (8 Pt 1): 1135-1138.
- 25- Witko-Sarsat V, Friedlander M, Capeillère-Blandin C, Nguyen-Khoa T, Nguyen AT, Zingraff J, Jungers P, Descamps-Latscha B. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. *Kidney Int.* 1996, 49 (5): 1304-1313.
- 26- Liu SX, Hou FF, Guo ZJ, Nagai R, Zhang WR, Liu ZQ, Zhou ZM, Zhou M, Xie D, Wang GB, Zhang X. Advanced oxidation protein products accelerate atherosclerosis through promoting oxidative stress and inflammation. *Arterioscler ThrombVasc Biol.* 2006, 26 (5): 1156-1162.
- 27- Nielsen F, Mikkelsen BB, Nielsen JB, Andersen HR, Grandjean P. Plasma malondialdehyde as biomarker for oxidative stress: reference interval and effects of life-style factors. *Clin Chem.* 1997, 43 (7) : 1209-1214.

239 28- Gutteridge JMC. Lipid peroxidation and antioxidants asbiomarkers of tissue damage.
240 Clinical Chemistry. 1995, 41 (12) 1819–1828.

241 29- Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative
242 damage in human disease. Clin Chem. 2006, 52 (4): 601-623.

243 30- Ohkawa H, Ohishi N, Yagi K, Assay for lipid peroxides in animal tissues by
244 thiobarbituric acid reaction, Analytical Biochemistry. 1979, 95 (2) 351–358.

245 31- Wong SH, Knight JA, Hopfer SM, Zaharia O, Leach CN Jr, Sunderman FW Jr.
246 Lipoperoxides in plasma as measured by liquid-chromatographic separation of
247 malondialdehyde-thiobarbituric acid adduct. Clin Chem. 1987, 33(2): 214-220.

248