

**Original article****MCP-1 (monocyte chemoattractant protein-1) concentrations in patients on hemodialysis. An observational study****V.D. Raikou*, D. Kyriaki***Department of Medicine - Propaedeutic, National & Kapodistrian University of Athens, School of Medicine, Greece.*

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ABSTRACT

Monocyte chemoattractant protein-1 (MCP-1) is the main representative of chemokines, whose the main function is to direct the migration of circulating leukocytes to sites of inflammation. The present study focuses on the intra- and interdialytic MCP-1 changes in patients on hemodialysis. We studied 76 stabled dialyzed patients (47 males / 29 females) and 24 healthy subjects. The treatment modalities which were applied were: regular hemodialysis (HD, n=34) and predilution hemodiafiltration (HDF, n=42) using synthetic high-flux dialysis membranes. Four blood samples were drawn (pre-hemodialysis, 2 hours, 4 hours and 44 hours after the beginning of the mean weekly dialysis session) and serum MCP-1 concentrations were measured using ELISA. MCP-1 levels were not found higher compared to control group (p=NS), but significantly changed over time (p<0.05). The hemodialysis modality or the material of used dialysis membranes did not influence the observed changes of MCP-1 over time (p=NS). In conclusion, MCP-1 serum concentrations in stabled patients on permanent hemodialysis treatment were significantly changed over time independently on the hemodialysis modality, despite the pre-dialysis MCP-1 mean value was similar to those in healthy subjects.

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1. Introduction

During the renal replacement therapies the circulating leukocytes of blood are activated and proinflammatory cytokines are produced including interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α) (Papayianni et al, 2002, Rousseau et al, 2000). This process is linked to complement activation (C3a, C5a), anaphylatoxin generated, bacterial derived fragments or endotoxins that may enter in the blood (Okusawa et al, 1988). The magnitude of the activation of cellular elements of blood and the cytokines production is associated to used dialysis membranes known as "biocompatibility".

The production of cytokines up-regulates dramatically the expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) on the cell surface, where they support the interaction of leukocytes and endothelial cells (Papayianni et al, 2002). Also, it has been shown that hemodialysis induces phenotypic changes in adhesion molecules expression on monocytes, which is continued during interdialytic period and influences the adherence to endothelial cells (Stoll and Bendszus, 2006, Jacobson et al, 2000).

MCP-1 (monocyte chemoattractant protein-1) is the main representative of chemokines, which are cytokines whose the main function is to direct the migration of circulating leukocytes to sites of inflammation (Blai Coll et al, 2007).

Chemoattractants stimulate leukocyte transmigration through the endothelium and may induce directional locomotion to atherosclerotic lesions. MCP-1 acts as a chemoattractant specific for monocytes and may promote migration of monocytes into the plaque after their initial adhesion to the endothelium, playing a particular role on the lesions of atherogenesis (Jacobson et al, 2000). The present study focuses on the intra- and interdialytic MCP-1 changes in patients on hemodialysis.

2. Materials and methods

2.1. Subjects

We studied 76 hemodialyzed patients who provided informed consent prior to participation in the present study, which was also approved by the ethics committee of the hospital.

47 men and 29 women participated in this study, on mean age 62.2 \pm 15 years old and 24 healthy subjects. Two modes of treatment were applied, regular hemodialysis (HD, n=34) and online- predilution haemodiafiltration (on-line HDF, n=42). The median time on hemodialysis was 5.0 \pm interquartile range 3-10 years. Patients with significant infection or malignancy were excluded from our study. The enrolled patients had a good status and they did not have interdialytic peripheral oedema or other characteristics of an inaccurate dry body weight. Per os medicines were including calcium channel blockers as antihypertensive drugs at the time of the study, the phosphate binders without intake of calcium, although any patient was receiving hypolipidemic medicines. The total of the enrolled patients were on recombinant human erythropoetin - α or - β therapy and 50% of the studied patients were on parenteral iron sucrose treatment.

The treatment was performed 3-times weekly with a dialysis time of 3.5-4 h per session, a filter of 1.5-2 m² surface area and a blood flow of 350-400ml/min. A bicarbonate-based ultrapure buffer dialysis solution was used with a dialysate flow rate of 500-600ml/min, a calcium concentration of 1.50-1.75mmol/L and a sodium concentration of 138-145mmol/L. The mean value of the convective volume in the HDF was 24.2 liters (17 – 40 liters). Low molecular weight heparin was exclusively used.

We used synthetic high-flux membranes, defined by a ultrafiltration coefficient >20 ml/h (Chauveau et al, 2005) with a material by polysulfone, ethynivinilalkooli, polyethersulfone and polyamid.

Dialysis dose defined by Kt/V for urea, which was calculated according to the formula of Daugirdas (Daugirdas, 1993). 20 studied patients disposed urine volume up to 100ml/day.

The primary renal diseases were hypertensive nephrosclerosis (n=25), chronic glomerulonephritis (n=23), polycystic kidney disease (n=9), diabetic nephropathy (n=7), and other/unknown (n=12).

2.2. Laboratory measurements

Blood was drawn just before the start of the mid-week dialysis session in a fasting state from the vascular access. In the end of the treatment the blood pump speed was reduced to <80ml/min and blood samples was obtained at 2 min postdialysis from the arterial dialysis tubing.

4 blood samples were received (pre-hemodialysis, 2 hours, 4 hours and 44 hours after the beginning of the mean weekly dialysis session) and serum MCP-1 levels were measured using enzyme linked immunoabsorbed

assay (ELISA, Alpco Diagnostics, Anachem, USA) with a reported interassay coefficient of variation below or equal to 7.4%. The samples at 2 hours and 4 hours after the start of dialysis session were corrected for changes in distribution volume of MCP-1, assuming that the change in bodyweight during the dialysis represents the change in extracellular volume due to ultrafiltration and that the distribution volume for MCP-1, is identical to the total extracellular volume and not only to the plasma volume. We supposed that the kinetic of MCP-1, as a middle molecule (9000 to 15,000 Da), is similar to beta-2 microglobulin kinetic, that is the known surrogate marker of middle molecule uremic toxins (Bergstrom and Wehle, 1987).

Normalized protein catabolic rate for dry body mass (nPCR) was calculated from the urea generation rate (Dougirdas, 1995) and body mass index (BMI) was obtained from height and postdialysis body weight.

2.3. Statistical analysis

Normal distributed values were expressed as mean \pm standard deviation or mean \pm standard error of mean as appropriate. Differences between mean values were assessed by using unpaired t-test for two groups. Data that showed skewed distributions were expressed as median value \pm interquartile range and were compared with Mann-Whitney U- test.

Repeated measures analysis of variance (ANOVA) was used to analyse change in MCP-1 serum concentrations over time and statistical significant differences were defined by paired t-test. Also, we examined the impact of dialysis modality and the material of used membranes on the changes of MCP-1 over time.

Analyses were carried out with SPSS 15.0 statistical package for Windows (SPSS Inc, Chicago, Illinois).

3. Results

Characteristics of the study population are listed in Table 1.

Table 1
Characteristics of the studied population, n=76 (47 males / 29 females).

	Minimum	Maximum	Mean/median	S.D/Interq range
age	24.00	87.00	62.2 /	15.01 /
Hemodialysis duration (years)	0.50	27.00	/ 5.0	/ 3-10
BMI (Kg/m ²)	18.9	32.9	24.4 /	3.03 /
Urine volume (ml / day)	100.00	500.00	/ 150	/ 100-300
KT/V for urea	1.20	2.01	/ 1.29	/ 1.25- 1.49
Normalized protein catabolic rate (nPCR)	1.12	4.31	2.43 /	0.57 /
MCP-1 (pg/ml)	45.58	689.41	258.27 /	137.95 /
Albumin (gr/dl)	1.40	4.60	/ 4.0	/ 3.8 – 4.2

The mean value of MCP-1 was not found higher in comparison to control group (p=NS, Figure 1), either significant difference was found among the following hemodialysis modalities (p=NS, Figure 2).

Indeed, we observed that the mean value of MCP-1 \pm SD of the second blood sample was significantly lower than the mean value of the pre-dialysis blood sample (194.96 \pm 107.38 vs 258.27 \pm 137.95, p=0.001). However, the mean value of the third blood sample did not differ from the first sample, either from the fourth blood sample. Nevertheless, the mean value of the third blood sample was significantly higher than the mean value of the second blood sample (270.89 \pm 159.78 vs 194.96 \pm 107.38, p=0.001). The mean value of the fourth blood sample was lower than the mean value of the first blood sample (228.66 \pm 135.53 vs 258.27 \pm 137.95, p=0.06) (Figure 3).

Controversially, we did not observe significant influence on MCP-1 changes over time by dialysis modality (p=NS, figure 4), or by the material of the used dialysis membranes (p=NS).

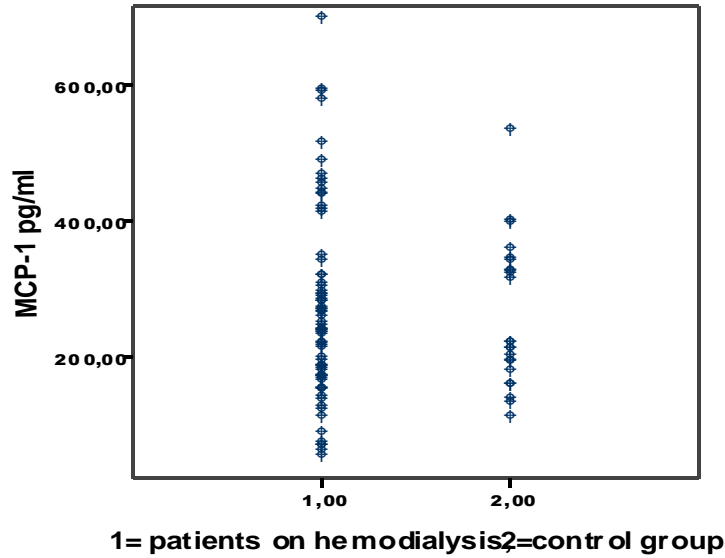


Fig. 1. Scatterplot for MCP-1 (pg/ml) serum concentrations comparatively between total patients and healthy subjects ($p=NS$).

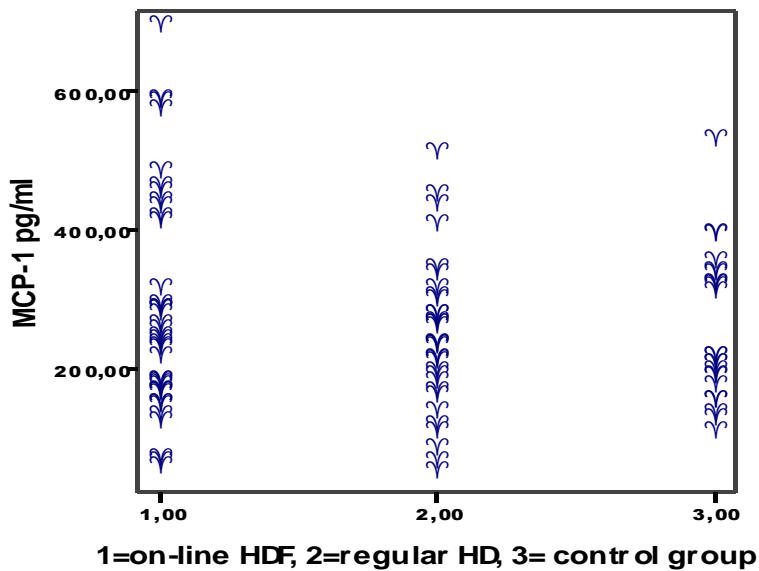


Fig. 2. Scatterplot for MCP-1 (pg/ml) serum concentrations comparatively between the patients on on-line hemodiafiltration, the patients on regular hemodialysis and healthy subjects ($p=NS$).

The repeated measures analysis showed significant change over time for MCP-1 serum concentrations ($p<0.05$) (Fig. 3).

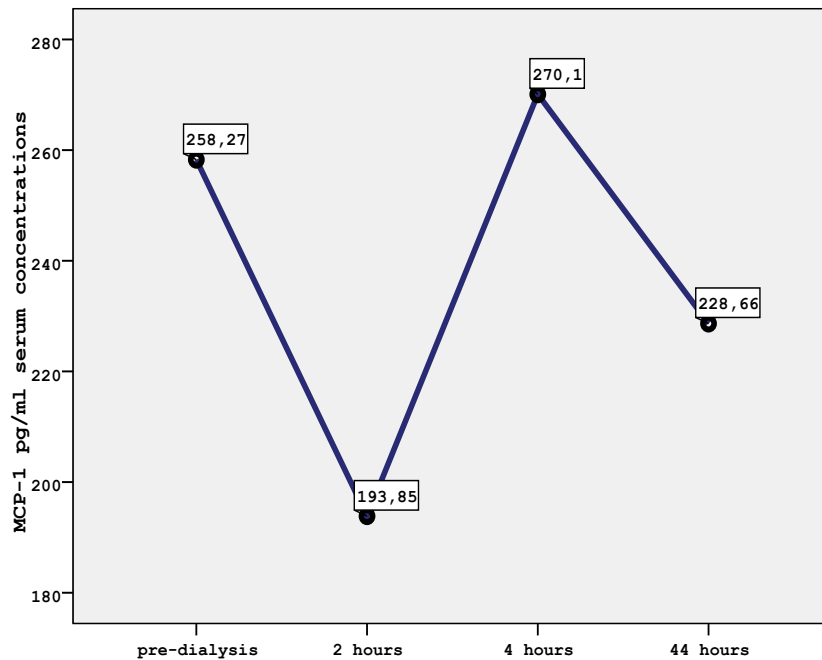


Fig. 3. Plot of linear model analysis for repeated measures of MCP-1 (pg/ml) serum concentrations over time and appearance of the mean values in each time point (pre-dialysis, 2 hours, 4 hours and 44 hours after the beginning of the mean weekly dialysis session) ($p < 0.05$).

4. Discussion

Different approaches have been reported of whether the uremic state affects MCP-1 concentrations. It has been already supported that the kidney plays an important role in the catabolism of molecules as MCP-1 and dialysis treatment may importantly influence the MCP-1 serum levels mainly depending on used dialysis membranes.

Some previous studies observed a significant increase in circulating MCP-1 levels in hemodialyzed patients treated either cellulosic or synthetic membranes, compared with control subjects (Papayianni et al, 2002, Rousseau et al, 2000, Jacobson et al, 2000). In contrary, other study using an *in vitro* method, reported significantly lower spontaneous production of MCP-1 from mononuclear cells of uremic patients, compared to healthy subjects and that hemodialysis using synthetic membranes normalized MCP-1 release from mononuclear cells which had been reduced by cuprophane treatment (Pertosa et al, 1998). However, the comparable levels reported in hemodialysis, peritoneal dialysis and pre-dialysis patients probably show that dialysis itself does not affect significantly their elimination (Bonomini et al, 1998).

In present study the serum concentrations of circulating MCP-1 were not significantly increased in hemodialyzed patients in comparison to healthy subjects, despite we used exclusively synthetic high-flux dialysis membranes. Also, the following hemodialysis modality did not seem to significantly impact on MCP-1 serum concentrations, in accordance to previous studies.

Additionally, in this study MCP-1 values were comparable with those reported in some previous studies (Mistrík et al, 2010), but with others studies were not. The different findings for MCP-1 data in patients with uremia, may suggest that the methods used for chemokines analysis have to be taken into consideration.

In the mean time, previous study reported significantly increased levels of MCP-1 in patients treated with erythropoietin (De Marchi et al, 1997). In contrast, other study reported that the treatment with erythropoietin in these patients decreased the levels of chemokines including MCP-1, when the patients were not in iron therapy (Pawlak et al, 2006). In this study, the total of included patients were on long-term erythropoietin treatment. Regarding the iron role, it has been shown using experimental model that parenteral iron sucrose and

iron gluconate treatment can induce MCP-1 generation, possibly via transcriptional events or increasing oxidative stress (Zager, 2005). However, in this study, despite the half of our patients was in parenteral iron sucrose treatment, MCP-1 levels were not found increased compared with healthy subjects.

Moreover, the increase of molecules as MCP-1 is mainly observed in increased oxidative stress conditions and inflammatory diseases (Cachofeiro et al, 2008). In this study, the enrolled patients were in a good status excluding the patients with active infections and this could contribute to similar MCP-1 levels between the patients and healthy subjects.

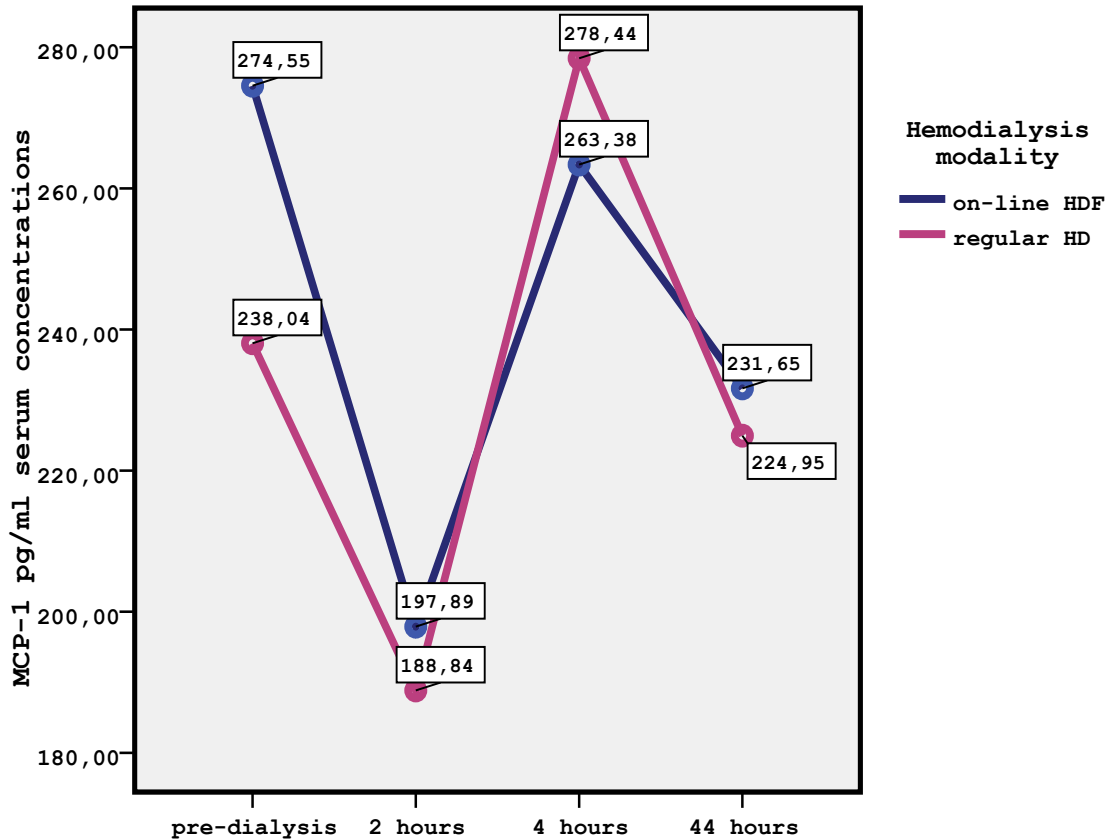


Fig. 4. Plots of linear model analysis for repeated measures of MCP-1 (pg/ml) serum concentrations comparatively in on-line HDF and regular hemodialysis over time and appearance of the mean values in each time point (pre-dialysis, 2 hours, 4 hours and 44 hours after the beginning of the mean weekly dialysis session) (p=NS).

On the other hand, in this study we observed statistically significant change of MCP-1 concentrations over time. Indeed, a significant decrease of the corrected for ultrafiltration volume MCP-1 levels was found two hours after the start of hemodialysis, although a such increase was found four hours from the beginning of hemodialysis session. Then, forty-four hours from the beginning of hemodialysis MCP-1 concentrations were lower than pre-dialysis concentrations.

The observed decrease of the corrected for ultrafiltration volume MCP-1 levels at the time point of two hours from the beginning of hemodialysis may due mainly to their adequate clearance by dialysis membrane.

Controversially, their increase at the end of dialysis session (four hours from the start of dialysis) may be attributed to changes of distribution volume of MCP-1 and it also is connected with rebound phenomenon.

Rebound phenomenon may be due to elevated release of plasma MCP-1 concentrations at that time point, to alterations of extracellular volume, or to a probable defect in clearance mechanisms at that time point. Indeed, a previous study showed that percentage of cytokine-positive cells was low prior to dialysis and then increased, supporting that dialysis may induce *de novo* cytokine synthesis (Rousseau et al, 2000). Also, the expression of cytokine-positive blood cells leaving the dialyzer was lower than that of cells entering it, due to trapping of activated cells by dialyzer depending on dialysis membrane. Moreover, some cells leaving the dialyzer were activated at the end of dialysis, suggesting that adhesion to membrane and anaphylatoxin generation and complement activation, induce cytokine production (Rousseau et al, 2000, Okusawa et al, 1988).

However, previously it has been reported stably increased MCP-1 levels during hemodialysis, in contrast to our study, where we observed significant MCP-1 decrease two hours after the start of dialysis. This difference may be connected to our different methodology, as the blood sampling was at different time points. Also, the modified dialysis membranes material by industries may play an important role on these different results.

Finally, forty-four hours from the start of dialysis we observed lower MCP-1 concentrations compared to pre-dialysis levels, even if non statistically significant, in accordance to other previous study, in which 8 hours after the beginning of hemodialysis MCP-1 concentrations were significantly lower than pre-dialysis levels (Jacobson et al, 2000). This finding could be attributed to regulated by hemodialysis immune response, which contributes to decreased MCP-1 generation, or to intradialytic elimination and post-dialytic redistribution of the molecule.

We could suggest that MCP-1 serum concentrations in hemodialysis patients are affected by its generation due to immunological intradialytic changes, in combination with the impact of intradialytic hydration status alterations and its clearance mechanisms.

5. Conclusion

The findings of the present study showed that MCP-1 serum concentrations in stable patients on permanent hemodialysis treatment were significantly changed over time independently on the following dialysis modality, despite the pre-dialysis MCP-1 mean value was similar to that of healthy subjects.

6. Limitations

The number of studied dialyzed patients is small and the impact of MCP-1 changes on the clinical situation of our patients may be not valid in present study. More patients need to conclude something about that.

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This study has not been connected by any financial or other conflict of interest.

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