RESEARCH ARTICLE

The Effect of Bacterial DNA and Hemodialysis Therapy Conditions on Interleukin 6 Levels Variation in End-stage Renal Disease Patients

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ABSTRACT

Hemodialysis (HD) is the most common therapy to treat end-stage renal disease (ESRD) patients. That is used to clear blood from metabolism end products and maintain water and electrolyte balance in patients' body when failed kidneys unable to do that. Patient's blood contact via dialysis filter (dialyzer) about 400 L of dialysis fluid that is mainly form of water and some electrolyte, this quantity of fluid contact can stimulate the production of pre-inflammatory markers due to carrying pathogens such as bacterial DNA fragments even in small quantity that can pass all filters to bloodstream stimulating production of IL-6. A total of 90 blood samples were collected from 45 patients under hemodialysis therapy in Baghdad Teaching Hospital, Gazy Alhariry general hospital, Al-khayal private hospital and Al-Muayad private hospital before and after 3 hours of HD therapy to detect IL-6 by ELISA technique. And from the same centers dialysis fluid and water samples were collected to detect bacterial DNA fragments.

PCR technique using 16s universal bacterial primer showed presence of bacterial DNA fragments in dialysis fluid and water used to prepare it.The ELISA readings showed a variety in IL-6 levels higher in patients with low ultrafiltration UF rate than its level in patients with high UF and generally, the level of IL-6 in patients treated in centers with DF preparation direct water distribution system were lower than its level in patients treated in centers with indirect water distribution systems.

Keywords: Dialysis fluid, Hemodialysis, High flux, Interleukin, Permeability, Reverse Osmosis, Ultrafiltration.

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INTRODUCTION

Chronic kidney disease (CKD) is a serious public health issue affecting people worldwide. The presence of kidney injury or a lower glomerular filtration rate (GFR), regarded as the greatest overall indicator of renal function, is used to characterize CKD.[1] There are 6 stages of kidney diseases related to GFR and the 6th stage is the end stage renal disease ESRD.[2] Patient in the ESRD as a matter of life or death, has two choices either go to kidney transplantation that demands a kidney doner[3] or go to hemodialysis.[4] Most ESRD patients prefer hemodialysis due to the risk of surgery regarding kidney transplantation and to avoid immunity-reducing drugs used to prevent new organ rejection and finding a kidney donor.[3-5]

Hemodialysis therapy was first known in 1943.[6] Since that time, many developments occurred to prevent its side effect such as immuno-compatibility of the dialyzer until the present with perfect results in dialyzer immune-compatibility.[6]

This study focused on some techniques and conditions of hemodialysis that effect changing in IL-6 level that indicates pre-inflammatory response against pathogens during hemodialysis such as reverse osmoses water RO distribution systems that is commonly used in two types direct water distribution that involve of RO distribution directly after purification and indirect RO distribution system which contain an RO storage tank before distribution to hemodialysis machines[7] and the other condition is the ultrafiltration UF rate which is differ from patient to patient according to the variation of patients kidney ability to filtrate water.[8] The UF is a mechanical power that force fluid to migrate from blood side to DF side of the dialyzer to the drain and that migration of fluid could carry IL-6 with.[9]

MATERIAL AND METHODS

Subjects

Forty-five patients were selected from two types of hemodialysis centers in Baghdad, these centers differ in water distribution systems (direct and indirect water distribution systems).

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Table 1: Primer size and sequence used to amplify bacterial 16*s* RNA gene.

Gene name	*Primer sequence (5→3)*	*Size bp*	*Ref.*
16*s* Forward	CCTACGGGAGGCAGCAG	560	[10]
16*s* Reverse	CCCGTCAATTCCTTTGAGTT		

22 patients from indirect and 23 from direct water distribution system centers in ages from 35 to 57 (mean 46) years old with different rate of ultrafiltration for each patient.

Samples

One milliliter of blood samples was collected from each patient before and after hemodialysis therapy to indicate the level of IL-6.

Waters and dialysis fluid samples were collected from hemodialysis machines used in hemodialysis therapy, 9 mL of each sample. These samples were replicated weakly from March 17th to April 21st 2021.

Molecular Method

Water and DF samples were treated in the same way to precipitate nucleic acid by using 9 mL of each and adding 0.9 mL of 3M, 5.2 pH Sodium acetate and after mixing well 4 mL of isopropanol were added to the mixture and then centrifuged at 14000 rpm for 30 minutes. The precipitated nucleic acids were transferred to PCR using universal bacterial primer Table 1.

Immunology Methods

Using ELIZA technique to determine human IL-6 to determine IL-6 before and after hemodialysis. All patients were connected to hemodialysis machines using high flux dialyzer size 18 manufactured by BBRAUN Germany

RESULTS AND DISCUSSION

The PCR products monitored on gel electrophoresis Figure 1 illustrate the presence of bacterial DNA in water as well as in DF.

A 2-ELISA test results for IL-6 detection in sera samples of hemodialysis patients show that all samples were positive with different concentrations Table 2. The study included samples before and after 3 hours of hemodialysis with different rate of ultrafiltration regarding each individual patient.

The results indicate that all sera samples containing IL-6 in different levels of concentration and that could be analyzed as followings:

Sera samples from indirect water distribution centers give a significant higher concentration of IL-6 in compare to samples from direct water distribution centers according to statistics analysis at ($p < 0.05$, $p = 0.005653$) and this higher concentration could be due to bacterial DNA fragments in DF of these centers due to chance of contamination in this type of water distribution by consisting of water storage tanks after RO production and from that tank the distribution to dialysis machine occurs by an extra water pump and all of that gives chance for contamination by bacteria that could die in RO due to their survive unsuitable osmotic conditions of RO and

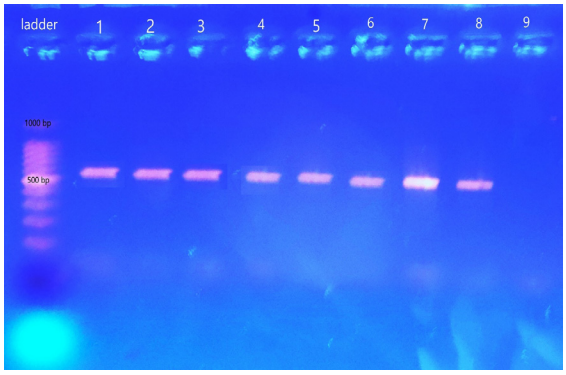

Figure 1: Electrophoresis results showing 16s RNA gene piece in water samples in numbers from 1 to 4 and DF samples from 5 to 8.

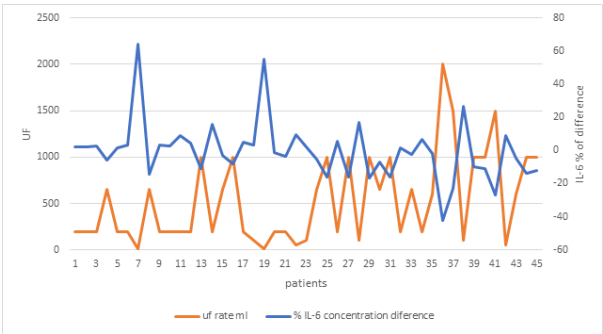

Figure 2: The relationship between ultrafiltration rate and the concentration of IL-6 in 45 patients after 3 hours of hemodialysis

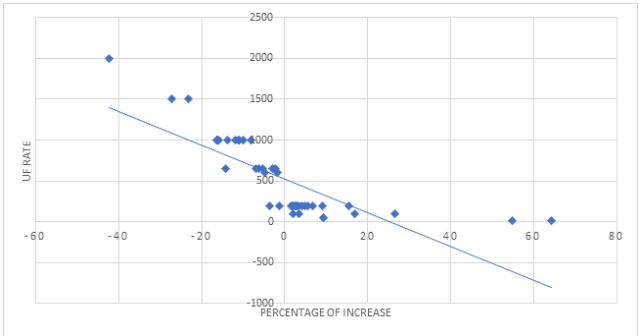

Figure 3: Statistic relationship between ultrafiltration rate and concentration of IL-6 that shows an inverse trend in total samples

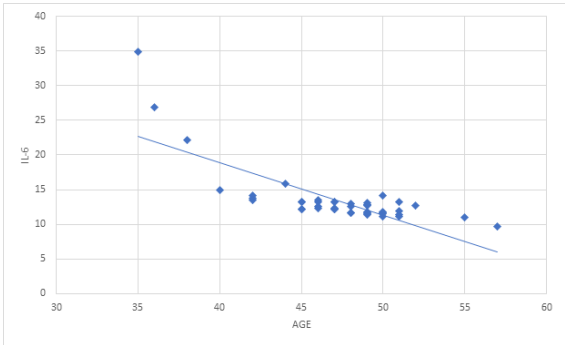

Figure 4: The relationship between patients age and IL-6 production in patients with hemodialysis.

Table 2: Interleukin 6 concentration pre and post-hemodialysis for patients from indirect and direct water distribution system with different rate of ultrafiltration

No.	*Patient ID*	*Age (years)*	*Water distribution type*	*Pre-dialysis conc. ng/L*	*Post-dialysis conc. ngL*	*% IL-6 *level Increase*	*UF rate mL*
1	2	55	direct	10.96	11.2	2.11	200
2	3	48	direct	11.62	11.85	1.99	200
3	4	47	direct	12.24	12.56	2.61	200
4	5	46	direct	12.24	11.49	-6.1	650
5	8	48	direct	12.57	12.8	1.84	200
6	14	49	direct	11.5	11.85	3.04	200
7	15	47	direct	12.21	20.07	64.34	10
8	16	46	direct	12.57	10.78	-14.21	650
9	18	45	direct	12.21	12.62	3.353	200
10	22	47	direct	12.15	12.47	2.63	200
11	23	45	direct	12.12	13.21	9	200
12	27	51	direct	11.14	11.61	4.2	200
13	30	50	direct	11.82	10.51	-11	1000
14	31	57	direct	9.72	11.22	15.51	200
15	32	51	direct	11.88	11.55	-2.79	650
16	34	50	direct	11.14	10.25	-8.04	1000
17	35	52	direct	11.59	12.15	4.81	200
18	36	48	direct	11.65	12.06	3.51	100
19	38	49	direct	11.35	17.58	54.84	10
20	42	50	direct	11.47	11.31	-1.34	200
21	43	49	direct	11.79	11.37	-3.57	200
22	44	51	direct	11.38	12.44	9.33	50
23	45	50	direct	11.62	11.85	1.99	100
24	1	45	indirect	13.28	12.59	-5.18	650
25	6	46	indirect	13.16	11.05	-16.	1000
26	7	42	indirect	13.81	14.58	5.54	200
27	9	36	indirect	26.85	22.59	-15.83	1000
28	10	45	indirect	13.19	15.41	16.82	100
29	11	44	indirect	15.8	13.21	-16.37	1000
30	12	40	indirect	14.9	13.9	-6.8	650
31	13	47	indirect	13.16	11.02	-16.27	1000
32	17	46	indirect	13.52	13.72	1.49	200
33	19	47	indirect	13.22	12.92	-2.29	650
34	20	49	indirect	12.8	13.66	6.67	200
35	21	49	indirect	12.74	12.53	-1.67	600
36	24	38	indirect	22.16	12.77	-42.35	2000
37	25	42	indirect	14.14	10.87	-23.12	1500
38	26	42	indirect	13.49	17.07	26.57	100
39	28	48	indirect	12.92	11.64	-9.91	1000
40	29	49	indirect	13.13	11.7	-10.89	1000
41	33	49	indirect	12.83	9.35	-27.09	1500
42	37	35	indirect	34.92	38.15	9.24	50
43	39	52	indirect	12.69	12.09	-4.72	600
44	40	51	indirect	13.25	11.43	-13.7	1000
45	41	50	indirect	14.17	12.47	-11.97	1000

*Negative values indicate decrease in level

then the bacterial DNA fragments can pass through all filters to patient blood to stimulate the immune system to produce more IL-6.[11]

There is an inverse relationship between ultrafiltration and the IL-6 concentration after 3 hours of dialysis therapy as shown in Figure 2 the number of IL-6 levels decrease with UF rate increase as an indication of IL-6 clearance ability. The statistics analyses at (p value <0.05, p value $=0.00433$) showed that there is a significant decrease of IL-6 concentration when ultrafiltration rate increases Figure 3 and that occur due to physical pressure difference on both sides of the dialyzer and that difference increases in direct relationship with ultrafiltration rate forcing fluid and particles in sizes of under the dialyzer cut off to migrate from blood side to dialysis fluid side and then to the drain. As well as that IL-6 size can be considered as middle-sized molecules.[12]

There is a noticeable difference in IL-6 concentration according to the patients ages as illustrated in figure 4. The study of age effect was done for samples before hemodialysis to prevent the effect of ultrafiltration on results and to study the accumulated concentration of IL-6 after the dialysis to the next dialysis therapy, which is regularly between 3 to 4 days.

The great difference shown in ages between 30 to 40 years old and not noticeable difference in ages between 40 to 60 years old. And that goes similar to the study of Sharif *et al.,* 2015 which studied the effect of hemodialysis patient's ages on IL-6.13

CONCLUSIONS

During hemodialysis, patients can gain pathogens such as bacterial DNA fragments that have the ability to pass all filters reaching the patients' and that induction increase when using an indirect water distribution system due to the chance of bacterial contamination.[5]

Ultrafiltration rate plays a role in decrease the concentration of IL-6 in blood to the dialyzers permeability and the physical power of ultrafiltration.

Younger patients show higher production of IL-6 in compare to older patients

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