ORIGINAL ARTICLE

Analysis of Water Used for Haemodialysis in Dialysis Centres, South East Nigeria: How Adequate?

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ABSTRACT

Background: *Standard water purity is one of the essential ingredients in achieving the goals of haemodialysis. However, water purity, though cardinal to the outcomes of haemodialysis, is probably the most neglected aspect of renal replacement therapy with haemodialysis.*

Methods: *A total of eight haemodialysis (HD) centres were studied. Water samples were analysed from three points: (a) water storage tank, (b) an outlet in the piping connection between the water storage tank and reverse osmosis (RO) machine and (c) an outlet piping between the RO machine and HD machine. Samples from A and B were referred to as pre-treated water, while samples from C were referred to as post-treated water. These samples were tested for aluminium, calcium and magnesium using the colorimeter; potassium and sodium using flame photometer; chloramines, nitrate, and free chlorine using colometric method. Water samples were also cultured in tryptone glucose extra agar at 37°C for 48 hours. Endotoxin analysis was done using limulus Amaeboctye assay.*

Results: *Borehole was the commonest source of water for haemodialysis, 63%. Treated water was tested for chemical and bacteriological contaminations every 3 months in 50% of the centres, every 6 months in 25% and rarely in 12.5%. One centre never tested their water. Combination methods were used in all the centres for water treatment. Mean concentration of aluminium (0.35 ± 0.06), chloramines (0.84 ± 0.88) and nitrate (2.54 ± 2.07) exceeded the Association for Advancement of Medical Instrumentation (AAMI) recommendation. The microbial counts were within the AAMI recommendation level.*

Conclusion: *Water purification in our environment is not optimal. This is cause for calls for serious concern.*

Keywords: Chronic kidney disease, haemodialysis, South East Nigeria, water treatment.

INTRODUCTION

The incidence of end-stage renal disease (ESRD) worldwide is consistently rising. The number of patients enrolled in the ESRD Medicare funded programme in USA has increased from approximately 10 000 in 1973 to 547 982 in December 2008 (1). The incidence of kidney diseases is higher in developing countries than in the industrialized world (2). By 2001, more than 1 million patients were reported worldwide to have received dialysis alone, with the number growing at an annual global average of 7% (3, 4).

Haemodialysis (HD) remains the most common modality of treatment in all the regions of the world, accounting for 60%–90% of renal replacement therapy (5). Dialysis patients are exposed to large volumes of water that is separated from patients' blood by the thin dialyser membrane. It is, therefore, important to subject water for HD use to proper treatment and monitoring in order to achieve and maintain the minimum chemical and bacteriological standard set by the Association for Advancement of Medical Instrumentation (AAMI) (6).

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South East Nigeria is one of the six geopolitical zones of Nigeria occupying the eastern part of the country. This is in the Ibo-speaking tribe. It constitutes approximately a quarter to a third of the Nigerian population currently estimated to be 158.2 million (7). There are 13 HD centres in the zone as at the time of this study, of which eight were functional.

The aim of this study, therefore, was to analyse the water used for HD in South East Nigeria for chemical and bacteriological content to determine if they met the standard recommendations.

MATERIALS AND METHODS

This study was carried out in eight busy functional HD centres located in Aba, Enugu, Onitsha, Orlu and Owerri, South East Nigeria. Four are located in tertiary health institutions; two in Christian mission hospitals, while two are in private hospitals.

Ethical clearance was obtained from relevant authorities of the participating centres.

Copies of a structured questionnaire were administered to the HD centres to assess the method of water treatment, water monitoring techniques, and source of feed water.

Three sets of the water samples were collected from each HD centre in sterile containers from (a) water storage tank, (b) an outlet in the piping connection between the storage tank and reverse osmosis (RO) (pre-treated water), and (c) after RO from an outlet in a pipe directly connected to HD machines (post-treated or treated water). The samples were collected on three different occasions at 3 months interval.

Based on the methods used in water treatment, the centres were divided into two groups: Group 1 and Group 2. Group 1 used filtration, activated charcoal and RO. Group 2 used filtration, softener, RO and ultraviolet (UV) irradiation.

The water samples for the bacteriological study were stored in ice pack and carried to the laboratory together with the samples for chemical analysis within 6 hours of collection. The samples for endotoxin analysis were stored in a refrigerator at 7°C (to prevent further bacterial growth) for 48 hours to allow for bulk analysis.

One hundred millilitres of water were collected from each sampling point after the water was allowed to run freely for 3 minutes. Using membrane filtration technique, the water samples were filtered using a sterile disc microfiltre, after which the membranes of the microfiltre were removed and laid in tryptone glucose extract agar contained in a pour plate. The samples were incubated at 37°C for 48 hours. The number of colony forming unit per plate was counted and recorded after 48 hours.

A semi-quantitative analysis of the endotoxin level was done using Limulus Amaebocyte assay method with sensitivity of 0.03 IU/mL. The detection and quantification of endotoxin was based on the colour change occurring after cleavage of a synthetic peptide complex. The colour change was read off in a colorimeter made by Sherwood, model DR 252 at 245 wavelength.

The water samples were taken to the South East Regional water laboratory located in Enugu for analysis. Aluminium, calcium and magnesium were analysed by a chartered chemist in the laboratory using a colorimetre made by Hatch model DR890 and titration method. Flame photometer, manufactured by Sherwood model number 410, was used to analyse potassium and sodium. Chloramines, nitrate and free chlorine were analysed using the colorimetric method.

Statistical analyses were performed using the SPSS version 17.0 statistical package for windows (SPSS Inc, Chicago, IL, USA). For continuous variables, mean values and standard deviation were calculated and the means compared using analysis of variance or Student's *t*-test as the case may be. Categorical variables were compared using the non-parametric test Chi-squared. All the tests were two-tailed, and $p < 0.05$ taken as statistically significant.

RESULTS

A total of eight HD centres were studied (Table 1). The centres were located in the urban cities. All were the central water treatment systems used. There was no automated water treatment control device.

Table 1: Demographic data of the haemodialysis centres

Centre no.	Location	Number of machines/centre
A	Enugu	4
B	Enugu	2
C	Enugu	3
D	Onitsha	4
E	Orlu	2
F	Owerri	2
G	Aba	5
H	Nnewi	2

The borehole was the commonest source of water supply, used solely in five (63%) centres (Table 2).

The feed water and treated water were tested for their bacteriological and chemical contaminants; feed water every 3 months in three centres (38%), every 6 months in two centres (25%), rarely in two centres (25%), and never in one (Table 3). Four of the centres (50%) tested their treated water every 3 months, three (38%) every 6 months and one (12%) every month (Table 3).

Table 2: Sources of water supply for haemodialysis

Source	Frequency (%)
Deep borehole	5 (63)
Tap water + tanker water supplies	1 (12)
Well water	1 (12)
Dam	1 (12)
Total	8 (100)

Table 3: Frequency of testing of feed water and treated water by HD centres

Frequency	No of centres	Percentage
Feed water		
Every 3 months	3	38
Every 6 months	2	25
Rarely	2	25
Never	1	12
Treated water		
Every month	1	12
Every 3 months	4	50
Every 6 months	3	38
Rarely	0	0

HD = haemodialysis.

Reverse osmosis and filtration methods were used as parts of the water treatment in all the centres. Group 1 (37.5% of the centres) used filtration, activated charcoal and RO. Group 2 (62.5%) used filtration, softener, RO and UV irradiation (Figure).

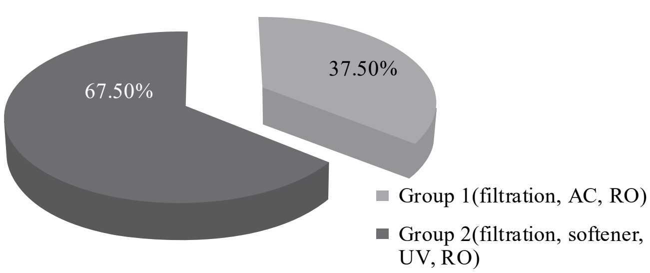

Figure: Distribution of water treatment methods in the centres. AC = activated charcoal; RO = reverse osmosis; UV = ultraviolet light.

The treatment method used in Group 2 gave a better clearance of magnesium, p-value = 0.041. There was no significant difference in the level of other chemicals tested in both groups (Table 4).

There was also no significant difference in the total coliform count and endotoxin level among the two groups (Table 5).

Table 4: Means of the differences in the value of chemical components pre- and post-treatment for the different treatment modalities

Chemical component (mg/L)	Treatment group 1 Mean ± SD	Treatment group 2 Mean ± SD	*F*	*p*
pH	− 0.37 ± 0.1	− 0.01 ± 0.7	0.488	0.640
Turbidity	0.56 ± 0.37	0.29 ± 0.28	1.219	0.370
Sulphate	45.1 ± 5.0	28.7 ± 15.1	1.111	0.399
Calcium	0.0	4.46 ± 3.46	2.431	0.183
Magnesium	0.19 ± 0.20	2.18 ± 1.39	6.464	0.041*
Chloramine	9.07 ± 3.66	32.26 ± 42.86	1.548	0.300
Chlorine	51.04 ± 29.39	21.57 ± 26.62	.807	0.497
Aluminium	0.14 ± 0.02	0.16 ± 0.02	.258	0.783
Fluoride	0.06 ± 0.00	0.10 ± 0.02	3.214	0.127
Nitrate	0.83 ± 0.66	7.88 ± 9.12	1.830	0.253
Sodium	1.89 ± 1.48	73.88 ± 103.40	1.836	0.252
Potassium	0.61 ± 0.56	6.07 ± 6.73	2.561	0.172
Total hardness	1.50 ± 0.71	18.00 ± 18.91	1.883	0.246

Table 5: Mean of the differences in value of microbial components pre- and post-treatment for the different treatment modalities

Microbial component	Treatment group 1 Mean ± SD	Treatment group 2 Mean ± SD	*F*	*p*
Total coliform count	122.50 ± 14.85	117.00 ± 50.91	0.761	0.515
Total endotoxin level	0.21 ± 0.05	0.15 ± 0.11	1.208	0.373

Back-flushing, addition of brine and rinsing were the generation methods used for the softener in 63% of the centres, while the remaining 37% used rinsing of the softener alone. The brand of RO machine used varied in different centres. Applied water engineering RO system was used in three centres (38%) owned by the government, Culligan RO system was used in two centres (25%) and unbranded RO system was used in the other centres (37%). All the centres carried out routine cleaning of the RO system.

The water was stored in polyvinyl chloride (PVC) storage tank in all the centres before treatment, six centres (75%) stored water in PVC tank after treatment before piping it to HD machine, while two centres piped their treated water directly to the HD machines. The shapes of the storage tanks were conical in four centres (50%), circular in 38% of the centres and square shaped in 12% of the centres. All the tanks had flat bottom and the delivery pipes were laid on the side of the tanks. The storage tanks were washed once in a month in 62% of the centres, once in 3 months in 25% of the centres and once in 6 months in 13% of the centres. Disinfection of storage tank was done with bleach in 62% of the centres, while 38% of the centres used hydrogen peroxide. The distributors of the piping systems in all the centres were made of PVC material and were connected on the sides of the tanks. Fifty per cent of the centres had more than five pipe joints, 38% had three pipe joints and one centre (12%) had two pipe joints. The distribution pipe systems were disinfected using bleach every 6 months in 62% of the centres, and in 38% of the centres, hydrogen peroxide was used in combination with other disinfectants in no regular fashion.

The mean concentration of sulphate, calcium, chloramines, chlorine, aluminium and nitrate in the feed water exceeded the AAMI recommendation, while the mean concentration of sodium, potassium, magnesium and fluoride were below the AAMI recommendation. After the treatment, the concentrations of chloramines and aluminium still remained above the AAMI recommendation, reaching even the toxicity level. The mean concentration of nitrate was above the recommended level but below the toxicity level after the treatment. The mean concentration of other elements investigated after the treatment was within the normal AAMI recommendation (Table 6).

There were statistically significant reductions in the levels of sulphate, chlorine, aluminium and fluoride after the treatment even though the levels of aluminium and chlorine were above the acceptable level (Table 6). The pH levels in all the centres were below 7.0. The turbidity and total hardness were less than 1 and 5, respectively, in the treated water.

The mean total coliform count and concentration of endotoxin level in both pre-treated and post-treated water in all the centres were below the recommended levels. When the water was subjected to the treatment, there were further significant reductions in the coliform count and endotoxin levels in the treated water, $p < 0.001$ (Table 7).

DISCUSSION

The treatment of the ESRD in Nigeria with HD has been in place since 1982, but the first HD centre in South East Nigeria was established in 1990.

Our study showed that the levels of sulphate, calcium, chloramines, aluminium and nitrate concentrations in the feed water were above the AAMI recommendations, whereas the levels of sodium, potassium and magnesium were within the recommended limits. This study also showed that the levels of all the tested chemical elements except chloramines and aluminium were

Table 6: Maximum recommended AAMI levels/mean values and mean differences of the chemical contaminants from all the centres pre- and post-treatment

Chemical component	Maximum AAMI level	Pre-treatment mean ± SD (mg/dL)	Post-treatment mean ± SD (mg/dL)	Mean diff. ± SD (mg/dL)	*T*	*p*
pH	–	6.70 ± 0.55	6.79 ± 0.41	– 0.09 ± 0.44	− 0.551	0.599
Turbidity	–	2.88 ± 4.42	0.07 ± 0.11	2.81 ± 4.45	1.784	0.188
Sulphate	100	115.45 ± 28.99	83.68 ± 23.05	31.77 ± 14.92	6.023	0.001
Calcium	2.0	3.34 ± 3.72	1.39 ± 1.64	1.94 ± 2.44	2.254	0.059
Magnesium	4.0	1.19 ± 1.29	0.43 ± 0.27	0.76 ± 1.04	2.067	0.078
Chloramines	0.10	12.77 ± 20.67	0.84 ± 0.88	11.93 ± 20.72	1.628	0.148
Chlorine	0.10	38.08 ± 22.78	0.47 ± 0.11	37.61 ± 22.73	4.681	0.002
Aluminium	0.01	0.35 ± 0.06	0.20 ± 0.06	0.15±0.03	15.027	< 0.001
Fluoride	0.20	0.15 ± 0.05	0.09 ± 0.04	0.07 ± 0.03	7.333	< 0.001
Nitrate	2.0	5.59 ± 6.28	2.54 ± 2.07	3.05 ± 4.63	1.862	0.105
Sodium	50	23.02 ± 50.67	3.42 ± 2.27	19.60 ± 51.49	1.077	0.317
Potassium	8.0	3.42 ± 5.81	1.55 ± 2.17	1.87 ± 3.65	1.449	0.191
Total hardness	–	12.24 ± 12.86	4.80 ± 4.14	7.45 ± 10.23	2.059	0.079

Table 7: Mean values and mean differences of the microbial components from all the centres pre- and post-treatment

Microbial component	Pre-treatment Mean ± SD	Post-treatment Mean ± SD	Mean diff ± SD	*t*	*p*
Total coliform count	156.88 ± 47.89	53.50 ± 29.98	103.38 ± 36.49	8.013	< 0.001
Total endotoxin level	0.38 ± 0.04	0.23 ± 0.07	0.15 ± 0.06	6.908	< 0.001

within the AAMI recommendations in the treated water. These findings contrasted with the findings of a similar multi-centre study done in Lagos, Nigeria (8) in which all the levels of the chemical contaminants in both the pre-treated and treated water exceeded the AAMI standard. The reason for this difference is not farfetched. Lagos has many industries that produce pollutants in various forms with the attendant contamination of the underground water. Before now, the sewage disposal in Lagos had been a very big problem to the extent that sewage commonly contaminates bore holes and wells. Lagos is also situated very close to the Atlantic Ocean, and, as a result, boreholes and wells are not usually dug deep before the water table is reached. The sea water is salty and contaminates the water table and the water supplies. Chemical water contaminants have been shown to increase morbidity and mortality in chronic HD patients (9).

In the South East of Nigeria, the major source of water is underground water such as well and the bore hole. Well water was the most predominant about two decades ago, but, with increasing government assistance and funding of water programmes by WHO and international donor agencies, deep bore holes and municipal water systems are now gaining prominence. Another source of water in the South East is tanker water supplies that get their water from the streams and the springs. This study showed that the major source of water for HD use in most of the centres was the bore hole (63%). This was because the HD centres some of which are profit-making outfits cannot rely on the erratic and inefficient municipal water system. Shallow boreholes and wells may be contaminated with chemicals, but the ones inspected in the centres where this work was carried out were deep bore holes about 300-m deep. They were located in the hospital where agricultural chemicals and sewage were less likely to contaminate them. This might be part of the reason why both the chemical and bacterial contaminants were within the limit of the AAMI recommendation. The result of this work is similar to the findings of Jose de Ribammar *et al* (10) in Brazil, a developing country, which showed that the bacterial level of dialysis water conformed to the AAMI standard, and that the major sources of feed water were municipal supply network and deep wells. The reports from developed countries showed that tap water regulated by safe water Acts were the major sources of water for HD applications (11–13). In our study, the level of contaminants did not differ much among the centres. This might be because most of them used deep boreholes as the source of water for haemodialysis. The small percentage of the centres that sourced water from municipal water supply had levels of contaminants close to that of the boreholes. Most of the pollutions by chemical contaminants reported in the literatures were episodic events (14, 15). Chloramine, which was above the AAMI recommendation in the feed water in our study, has been reported to be associated with dialysis-induce haemolytic anaemia (16). So, there is the need for the regular monitoring of the levels of contaminants in our feed water.

The AAMI recommends that feed water should be tested for both bacterial and chemical contamination once every month. No centre in this study met this recommendation. The best, only 35% of the centres, tested their feed water for bacterial and chemical contamination every 3 months. The frequency of testing water for HD use is better in developed countries despite the fact that their water source is the municipal water system that is strictly regulated. Pizzarelli *et al* (9) showed that 29% of HD centres in one region of Italy tested HD water every month, 14% every 2 months, 37% every 3 months, 4% every 4 months, 12% every 6 months and 4% yearly.

Our study also showed that the centres used different types of treatment modalities for their HD water. These included RO and filtration which were used by all the centres, ion-exchange, softeners, UV irradiation and activated charcoal. It should be noted that though there were differences in the reduction in the levels of the chemical contaminants in the different combinations employed, Table 4, it was only the reduction of magnesium in group1 (filtration, activated charcoal and RO) that was statistically significant, $p = 0.04$. Reverse osmosis was used in all the treatment combinations, and this could be responsible for the lack of statistically significant differences in the level of chemical contaminants between the two treatment groups. The studies where RO alone was used had shown very good reduction in the levels of chemical contaminants (17). While all the centres in this study employed RO, a study in USA by Takers *et al* (18) showed that RO was used alone in 55% of HD centres and in combination with the deionizer in 23% of the centres. No centre in our study used the deionizer. This might be the reason why aluminium remained high even after water treatment. Single methods of water treatment appeared to be favoured more in developed countries.

The levels of chloramine and aluminium concentration remained above the AAMI limit in our study. These chemicals can cause both acute and chronic toxicities in HD patients. The combination of the methods is expected to produce quality water for HD use especially in areas where feed water does not receive any form of treatment. Even though the use of combination methods should be encouraged in developing countries, care should be taken in the selection of the methods. This is because some combinations could actually lead to dismal outcome (19). Softeners and deionizers if not replaced or regenerated, could serve as good culture medium for the growth of microorganisms.

Five centres (63.5%) used UV irradiation as a part of their treatment modalities. In all the centres where UV irradiation was used, it was placed as the last step in the purification system. The disadvantage of this is the possible clogging of the UV filament, which could lead to the reduction in the irradiation dose reaching the microorganisms. AAMI guideline recommended that the UV filament should be cleaned routinely; there should be daily recording of UV dose and the lamp should be changed every year and disposed of, after 8000 hours of usage. Ultraviolet irradiation was newly installed and was not a part of the original water treatment system in three of the five centres. In the remaining centres, UV irradiation had been in use for more than 2 years and one of the centres has a trained technician in its employment. This centre cleans its UV filament every 3 months, which is in conformity with the AAMI recommendation. Frequent cleaning of the UV filament is very important because the effect of the UV system occurs only at the point of irradiation and thus does not sterilize the distributing system so that any organism that escapes the irradiation point could go on multiplying with increasing consequences.

There was a 100% use of RO in this study. This was also the finding in the study done by Rotimi in the Lagos area of Nigeria (8). RO has the ability to remove bacteria and endotoxin from water. It has a rejection rate of 90%–95% for univalent ions and 95%–99% rejection rate for divalent ions. All the centres had rejection rate > 90%. There was a remarkable reduction in the level of calcium, aluminium, chloramines and nitrate concentrations after the treatment. Even though RO was used by all the centres, the levels of aluminium and chloramines still remained above the AAMI limits after the treatment. This might be accounted for by the minimal rejection level of the RO system and the non-inclusion of the de-ionization method in the water purification system. The reports from other studies showed that RO significantly reduced the levels of the contaminants (19, 20). However, Lapierre and Bambauer, in separate studies, showed that when RO alone was used, the endotoxin level still remained higher than the recommended level (21, 22). To achieve the maximum effect of RO, routine and regular disinfection and monitoring should be observed.

Water was stored in the tanks made of PVC in all the centres before the treatment. The most ideal material for water storage tank in the HD water system is stainless steel. PVC was commonly used due to its relative low cost. Studies in Greece, Italy and USA reported that PVC is the commonly used material for storage tanks and the distributing piping systems (23–25). Our study showed that even though the shape of the storage tanks was conical in four centres, they all had flat bottom. This is not in line with the AAMI guidelines, which recommended tanks with conical bottom with pipe connected to the bottom to make sure dead spaces are eliminated (26). Half of the studied HD centres have more than five pipe joints, which are more than the AAMI recommendation of two pipe joints. Though the levels of endotoxin and the coliform count in all the centres were below the AAMI limits, there is still the need for these HD centres to adhere to the international standard of less than two pipe joints to improve further the quality of the HD water. Biofilm formation is less when the pipe joints are less than two. Biofilm when formed leads to release of bacterial compounds which cause chronic inflammatory state in the HD patients. Biofilm is also resistant to most methods of disinfection; so, the prevention of its formation is the ideal.

This study showed that disinfection in the HD centres fell below the AAMI recommendation of once every month (26). Sixty-three per cent of the centres disinfected their water treatment and distribution system once every 6 months. This might not have constituted an extra danger to the HD patients since the bacteria count and endotoxin levels in the treated water were below the AAMI level. However, it is important to adhere to the frequent disinfection to avoid the development of biofilm in the distribution systems. The use of automated water treatment control device was not practised in any of the HD centres. Automated water treatment control device is important to prevent water stagnation during the night and weekends when the centres might not be working. As good as this may be, the device may be difficult to operate in our environment because of the erratic power supply.

In conclusion, the water treatment for HD in our centres is suboptimal. Therefore, concerted efforts should be made to keep to the recommended standards.

We recommend the provision of pipe-borne water that conforms to the international standards, adherence to the AAMI minimum standard for the purity of water for HD in our environment, deep borehole to be used as a source of HD and municipal water supplies only as an alternative. Such boreholes should be sited away from human and animal waste to avoid contamination. The number of pipe joints should not be more than two to minimize the formation of biofilm in the piping system. The regeneration process of the ion-exchange methods should include back flushing, rinsing and the addition of brine to increase the efficiency of the methods. The de-ionization method should be included in the water treatment to reduce the aluminium levels, and double RO method placed in sequence should be employed to achieve ultrapure water for HD applications. Furthermore, the training of HD technicians on the importance of regular maintenance of water purification systems, the formation of the committee by the Nephrology Association of Nigeria that will monitor and ensure that minimum water quality standard similar to the recommendation of AAMI is maintained and further study to evaluate the common microbial organisms implicated in microbial contamination of water for the effective HD applications in our dialysis centres should be pursued vigorous and continuously.

LIMITATIONS OF THE STUDY

Lead, copper and zinc were not analysed because the only atomic absorption photometry in the area broke down at the time of this study. Individual microorganism count was not possible because of the difficulty in sourcing the reagents, needed for their detection.

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