Analysis of Water Used For Haemodialysis In Dialysis Centers, 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 outcome of haemodialysis is probably the most neglected aspect of renal replacement therapy with haemodialysis.

Methods: A total of eight haemodialysis centers 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 machine and C) an outlet piping between the reverse osmosis machine and haemodialysis machine. Samples from A and B were referred to as pretreated water while samples from C were referred to as post treated water. These samples were tested for aluminum, calcium and magnesium using 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 ºC 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 three months in 50% of the centers, every six months in 25% and rarely in 12.5%. One centre never tested their water. Combination methods were used in all the centers for water treatment. Mean concentration of aluminum (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 AAMI recommendation level.

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

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

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INTRODUCTION

The incidence of end-stage renal disease (ESRD) worldwide is consistently rising. The number of patients enrolled in the end stage renal disease Medicare funded programme in United States of America (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 one million patients were reported worldwide to have received dialysis alone, with the number growing at an annual global average of 7% (3, 4).

Haemodialysis remains the most common modality of treatment in all 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 patient's blood by the thin dialyzer membrane. It is therefore, important to subject water for haemodialysis use to proper treatment and monitoring in order to achieve and maintain the minimum chemical and bacteriological standard set by Association for Advancement of Medical Instrumentation [AAMI] (6).

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 haemodialysis centers in the zone as at the time of this study, of which eight were functional.

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

MATERIAL AND METHODS

This study was carried out in eight busy functional haemodialysis centers 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 centers. Structured questionnaires were administered to the haemodialysis centers to assess method of water treatment, water monitoring techniques and source of feed water.

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

Based on methods used in water treatment, the centers were divided into two groups- group1 and group 2. Group 1 used filtration, activated charcoal and reverse osmosis. Group 2 used filtration, softener, reverse osmosis and ultraviolet irradiation.

Water samples for bacteriological study were stored in ice pack and carried to the laboratory together with the samples for chemical analysis within six hours of collection. Samples for endotoxin analysis were stored in refrigerator at 7 $^{\circ}$C (to prevent further bacterial growth) for 48 hours to allow for bulk analysis.

100 mLs of water were collected from each sampling point after water was allowed to run freely for three minutes. Using membrane filtration technique, water samples were filtered using a sterile disc microfilter, after which the membranes of the microfilter were removed and

laid in tryptone glucose extract agar contained in a pour plate. Samples were incubated at 37 °C for 48 hours. The number of colony forming unit (cfu) per plate were counted and recorded after 48 hours.

Semi-quantitative analysis of endotoxin level was done using Limulus Amaebocyte assay method with sensitivity of 0.03 IU/mL. Detection and quantification of endotoxin was based on color change occurring after cleavage of a synthetic peptide complex. The color change was read off in colorimeter made by Sherwood; model DR 252 at 245 wave length.

Water samples were taken to South-east Regional water laboratory located in Enugu for analysis. Aluminum, Calcium and magnesium were analysed by a chartered chemist in the laboratory using colorimeter 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 colorimetric method. Statistical analyses were performed using the SPSS version 17.0 statistical package for windows (SPSS Inc, Chicago, IL). For continuous variables, mean values and standard deviation were calculated and the means compared using analysis of variance or student *t*-test as the case may be. Categorical variables were compared using the non-parametric test Chi-squared. All tests were two-tailed and $p < 0.05$ taken as statistically significant.

RESULTS

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

Table 1: Demographic data of the haemodialysis centers

Center No.	**Location**	**Number of machines/center**
A	Enugu	4
B	Enugu	2
C	Enugu	3
D	Onitsha	4
E	Orlu	2
F	Owerri	2
G	Aba	5
H	Nnewi	2

Borehole was the commonest source of water supply, used solely in five (63%) centers (**Table 2**).

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)**

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

Table 3: Frequency of testing of feed water and treated water by haemodialysis centers

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

Reverse osmosis and filtration methods were used as part of water treatment in all the centers. Group1 (37.5% of the centers) used filtration, activated charcoal and reverse osmosis. Group2 (62.5%) used filtration, softener, reverse osmosis and ultraviolet irradiation (Fig 1).

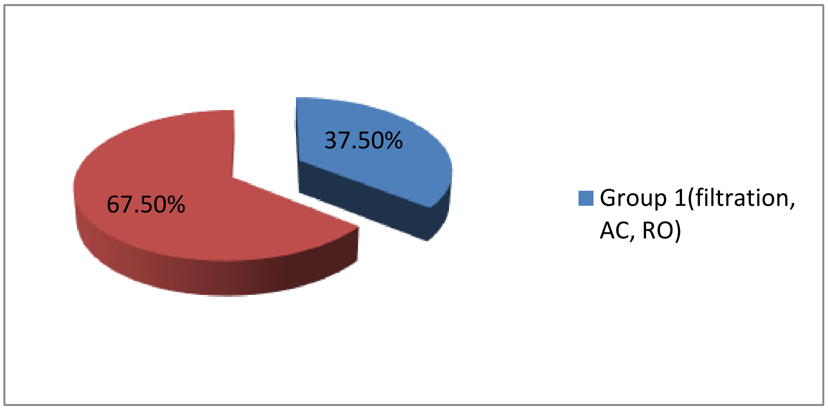

Figure 1: Distribution of water treatment methods in the centers. AC – activated charcoal, RO – reverse osmosis, UV – ultraviolet light.

Treatment method used in group2 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.

Table 4: Means of the differences in 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	0.807	0.497
Aluminium	0.14 ± 0.02	0.16 ± 0.02	0.258	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

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

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 softener in 63% of the centers while the remaining 37% used rinsing of softener alone. The brand of RO machine used varied in the different centers. Applied Water engineering RO system was used in three centers (38%) owned by government, Culligan RO system was used in two centers (25%)

and unbranded RO system was used in the other centers (37%). All the centers carried out routine cleaning of the RO system.

Water was stored in poly vinyl chloride (PVC) storage tank in all the centers before treatment, six centers (75%) stored water in PVC tank after treatment before piping it to haemodialysis machine while two centers piped their treated water directly to the haemodialysis machines. The shape of the storage tanks were conical in four centers (50%), circular in 38% of the centers and square-shaped in 12% of the centers. All the tanks had flat bottom and the delivery pipe were laid on the side of the tanks. The storage tanks were washed once in a month in 62% of the centers, once in three months in 25% of the centers and once in six months in 13% of the centers.

Disinfection of storage tank was done with bleach in 62% of the centers while 38% of the centers used hydrogen peroxide. The distributors of piping systems in all the centers were made of PVC material and were connected on the sides of the tanks. Fifty per cent of the centers 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 six months in 62% of the centers and in 38% of the centers hydrogen peroxide was used in combination with other disinfectants in no regular fashion.

The mean concentration of sulphate, calcium, chloramines, chlorine, aluminum 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 treatment, the concentration of chloramines, and aluminum still remained above AAMI recommendation, reaching even the toxicity level. The mean concentration of nitrate was above the recommended level but below the toxicity level after treatment. The mean concentration of other elements investigated after treatment was within normal AAMI recommendation, Table 6.

Table 6: Maximum recommended AAMI levels/Mean values and mean differences of the chemical contaminants from all the centers 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
Aluminum	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

There was statistically significant reduction in the levels of sulphate, chlorine, aluminum and fluoride after treatment even though the levels of aluminum and chlorine were above the acceptable level Table 6. The pH level in all the centers were below 7.0. The turbidity and total hardness were less than one and five respectively in treated water.

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

Table 7: Mean values and mean differences of the microbial components from all the centers 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

DISCUSSION

Treatment of end-stage renal disease in Nigeria with haemodialysis has been in place since 1982 but the first haemodialysis centre in South-East Nigeria was established in 1990.

Our study showed that the levels of sulphate, calcium, chloramines, aluminum and nitrate 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 levels of all the tested chemical elements except chloramines and aluminum were 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 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 which produce pollutants in various forms with the attendant contamination of underground water. Before now the sewage disposal in Lagos has 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, the boreholes and wells are not usually dug deep before water table is reached. The sea water is salty and contaminates water table and water supplies. Chemical water contaminants have been shown to increase morbidity and mortality in chronic haemodialysis patients (9).

In the south-east of Nigeria the major source of water is underground water such as well and bore hole. Well water was the most predominant about two decades ago but with increasing government assistance and funding of water programmes by World Health Organization (WHO) and international donor agencies, deep bore holes and municipal water systems is now gaining prominence. Another source of water in the South East is tanker water supplies which get their water from streams and springs. This study showed that the major source of water for haemodialysis use in most of the centers was bore hole (63%). This is because the haemodialysis centers 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 centers where this work was carried out were deep bore holes about 300 meters deep. They were located in the hospital where agricultural chemicals and sewage are less likely to contaminate them. This may be part of the reason why both the chemical and bacterial contaminants were within the limit of 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. Reports from developed countries showed that tap water regulated by safe water Acts were the major source of water for haemodialysis applications (11–13). In our study, the level of contaminants did not differ much among the centers. This may be because most of them used deep boreholes as source of water for haemodialysis. The small percentage of centers 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 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 need for regular monitoring of the level in our feed water.

Association for Advancement of Medical Instrumentation 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 centers tested their feed water for bacterial and chemical contamination every three months. The frequency of testing water for haemodialysis use is better in developed countries despite the fact that their water source is municipal water system that is strictly regulated. Pizzarelli *et al* (9) showed that 29% of haemodialysis centers in one region of Italy tested HD water every month, 14% every two months, 37% every three months, 4% every four months, 12% every six months and 4% yearly.

Our study also showed that the centers used different types of treatment modalities for their HD water. These include, reverse osmosis and filtration which were used by all the centers, ion-exchange, softeners, ultra violet irradiation and activated charcoal. It should be noted that though there were differences in reduction of 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 reverse osmosis) 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. Studies where reverse osmosis alone was used had shown very good reduction in the levels of chemical contaminants (17). While all the centers in this study employed reverse osmosis, a study in USA by Takers *et al* (18) showed that reverse osmosis was used alone in 55% of HD centers and in combination with deionizer in 23% of centers. No center in our study used deionizer. This may be why aluminum remained high even after water treatment. Single methods of water treatment appeared favoured more in developed countries.

Levels of chloramine and aluminum remained above the AAMI limit in our study. These chemicals can cause both acute and chronic toxicities in haemodialysis patients. Combination of 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 methods. This is because some combinations could actually lead to dismal outcome (19).

Softener and deionizer if not replaced or regenerated could serve as good culture medium for the growth of micro-organisms.

Five centers (63.5%) used ultra-violet irradiation as part of their treatment modalities. In all the centres where ultraviolet light (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 reduction in irradiation dose reaching the microorganisms. Association for Advancement of Medical Instrumentation 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 light irradiation was newly installed and was not part of the original water treatment system in three of the five centers. In the remaining centers UV irradiation has been in use for more than two years and one of the centers has a trained technician in its employment. This center cleans its UV filament every three months which is in conformity with AAMI recommendation. Frequent cleaning of the UV filament is very important because the effect of the UV system occur 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 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). Reverse osmosis 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 remarkable reduction in the level of calcium, aluminum, chloramines and nitrate after treatment. Even though, RO was used by all the centres, the levels of aluminum and chloramines still remained above AAMI limits after treatment. This may be accounted for by minimal rejection level of the RO system and non inclusion of de-ionization method in the water purification system. Reports from other studies showed RO significantly reduced the level of 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 maximum effect of RO, routine and regular disinfection and monitoring should be observed.

Water was stored in tanks made of polyvinyl chloride (PVC) in all the centres before treatment. The most ideal material for water storage tank in HD water system is stainless steel. Polyvinyl chloride 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 is conical in four centres, they all had flat bottom. This is not in line with 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 haemodialysis centres have more than five pipe joints which is more than AAMI recommendation of two pipe joints. Though the level of endotoxin and the coliform count in all the centres were below AAMI limits, there is still need for these haemodialysis centers to adhere to international standard of less than two pipe joints to improve further the quality of 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 prevention of its formation is the ideal.

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

In conclusion, water treatment for HD in our centers is suboptimal. Concerted effort should be made to keep to recommended standards.

We recommend provision of pipe borne water which conforms to international standards, adherence to AAMI minimum standard for purity of water for haemodialysis in our environment, deep borehole to be used as source of Haemodialysis 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, regeneration process of the ion-exchange methods should include back flushing, rinsing and addition of brine to increase the efficiency of the methods, de-ionization method should be included in water treatment to reduce aluminum levels, double reverse osmosis method placed in sequence should be employed to achieve ultrapure water for haemodialysis applications, training haemodialysis technicians on the importance of regular maintenance of water purification systems, formation of 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, further study to evaluate the common microbial organisms implicated in microbial contamination of water for haemodialysis application in our environment.

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.

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