Methaemoglobinemia and haemolysis associated with hydrogen peroxide in a paediatric haemodialysis centre: a warning note

Miriam Davidovits¹, Ayala Barak², Roxana Cleper¹, Irit Krause¹, Zahava Gamzo¹ and Bella Eisenstein¹

¹Nephrology Clinic and Dialysis Unit, Schneider Children’s Medical Center of Israel, Petah Tikva and Sackler School of Medicine, Tel Aviv University, Tel Aviv and ²Aylab Ltd, Tel Aviv, Israel

Abstract

Background. Haemodialysis exposes patients to contaminants in the dialysate. The AAMI standards deal only with two disinfectants, chlorine and chloramine. We report an event of methaemoglobinemia and haemolysis related to an unsuspected disinfection agent.

Methods. Nine children aged 3–17 years undergoing dialysis after reconstruction of our paediatric dialysis unit developed methaemoglobinaemia of 3.1–11%, with a mean reduction in haemoglobin levels of 11.9 ± 5.9% (P < 0.001). Air bubbles were noted in the bloodlines. The water treatment system (WTS) of the dialysis unit is disinfected when necessary by adding concentrated hydrogen peroxide (HP) to the storage tank and circulating it through the re-circulation loop with draining and subsequent flushings. Total chlorine analysis of the water is performed by DPD-iodide colorimetric method.

Results. Dialysis water testing yielded a high chloramine concentration in the storage tank and points-of-use stations (3.08 and 2.06 p.p.m., respectively). However, this finding was not true for the tap water, and it also failed to explain the air bubbles in the dialysis tubing. The concentration of free chlorine was within the recommended range. Further investigation revealed that the WTS was disinfected by the service company during remodelling of the unit, without notification of the hospital staff. Since the DPD-iodide test is not specific, and in effect detects not only total chlorine, but all oxidants capable of oxidizing iodide, we assumed the culprit was residual HP that was inadequately flushed from the water system.

Conclusions. HP used for disinfection of the WTS can pose a serious dialysis risk if not flushed out properly.

Total chlorine analysis should be performed before every dialysis session, and positive results should prompt further work-up for other oxidants. The clinical staff must always be involved in decisions regarding any intervention in the dialysis water system.

Keywords: children; haemodialysis; haemolysis; hydrogen peroxide; methaemoglobinaemia

Introduction

The ancient Latin saying *Aqua turbida non lavat* (dirty water does not wash clean) is particularly relevant to haemodialysis patients. The vast quantity of water in the dialysate originates from drinking water. Any contaminants present in the water come into close contact with the patients' blood within the dialyser across a thin non-selective membrane that has none of the 'wisdom' of the gastrointestinal tract. Moreover, owing to their compromised renal function, haemodialysis patients lack the ability to excrete toxins efficiently, such that alterations in the chemical composition of the water may cause acute and chronic complications. Adverse effects have been reported in patients exposed to dialysis water containing high concentrations of aluminium, zinc, fluoride, copper, calcium or magnesium, sodium, nitrate, chloramine and sodium azide [1]. Haemodialysis patients also risk exposure to the chemicals used for the periodic disinfection of the fluid pathways of the water treatment system (WTS) [2].

In 1981, the Association for the Advancement of Medical Instrumentation (AAMI) established comprehensive water quality standards for the maximal concentration of minerals and heavy metals [3]. Most WTSs are constructed to provide water that meets the recommended standard. However, in the absence of appropriate treatment of the system with regular and stringent monitoring, the water used for preparing
dialysate can pose a serious and even fatal risk. Furthermore, the AAMI standards concern mostly inorganic agents; chlorine and chloramine are the only disinfectants listed, with suggested maximal levels of 0.5 and 0.1 p.p.m., respectively.

We report on an outbreak of methaemoglobinaemia and haemolysis in a paediatric dialysis unit due to contamination of the dialysate with hydrogen peroxide (HP).

**Subjects and methods**

The Hemodialysis Unit of Schneider Children’s Medical Center of Israel was opened in October 1995 with five stations and enlarged to 11 stations in December 1997. At the completion of reconstruction, nine patients aged 3–17 years were being treated in the unit. Dialysis was performed with Centry 3 and Gambro AK 100 machines and hollow fibre polysulphon dialysers (Fresenius). No reuse was practiced. At the beginning of the first dialysis session in the remodelled unit, air bubbles were noted in the bloodlines. Assuming that the problem was in the tubing sets or dialysers, we changed the equipment. However, the problem persisted. Subsequently, one patient turned grey and her blood in the tubing turned chocolate brown. Methaemoglobinaemia was suspected and confirmed by a high level of methaemoglobin (11%) in this patient. All haemodialysis treatments were immediately stopped; blood tests for haemoglobin, methaemoglobin and haptoglobin were taken before disconnection. The one patient with hypoxic symptoms and anaemia was immediately treated with methylene blue. The children were transferred to another hospital for continuation of dialysis.

**Chlorine analysis of water**

Chlorine and chloramine in the water are analysed by DPD-iodide colorimetric method [4]. DPD (N,N-diethyl-P-phenilenediamine) is oxidized instantly by free chlorine to a relatively stable free radical producing a red colour (Wurster dye). Absorption is measured by colorimeter at 530 nm. Because chloramines react slowly with DPD, they are quantified by the subsequent addition of potassium iodide. The iodide ion acts catalytically causing colour production by monochloramine and dichloramine. The iodide-DPD test method is typically used for measuring total chlorine, which is the sum of free (chlorine reacting with DPD directly) and combined (chlorine derivatives which react with DPD only in the presence of iodide).

**Method of disinfection**

To disinfect the water, the reverse osmosis unit is shut off and the UV light sources are disconnected. HP 30% is added directly to the storage tank and diluted with dialysis water to a final concentration of 1%. The HP is circulated in the

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**Fig. 1.** Scheme of the WTS of the dialysis unit.
re-circulation loop for 2 h. Thereafter, the circulation pump is stopped, the tank is emptied, the reverse osmosis system is switched on and the storage tank is refilled with fresh dialysis water. The re-circulation pump is operated for half an hour. The storage tank and re-circulation loop are then drained by opening all the point-of-use stations. Following several flushing cycles, a test for oxidative substances is performed by the DPD method at the points-of-use stations. Additional flushing cycles are performed until the residue measures <0.1 p.p.m.

Statistics
The data were analysed using the Student’s *t*-test (paired). A *P*-value of < 0.05 was considered significant. Correlations were calculated using Spearman’s method for non-parametric data.

Results
All patients showed methaemoglobinaemia with levels ranging from 3.1 to 11% (normal <1%), and a mean reduction in haemoglobin levels of 11.9 ± 5.9% (*P* < 0.001) from the last measured value. A decrease in haptoglobin level (normal >50 mg/dl) was noted in two cases (Table 1). The methaemoglobin levels were positively correlated with dialysis duration (*r* = 0.8). There was no correlation between duration of dialysis and decrease in haemoglobin levels.

The findings of methaemoglobinaemia and haemolysis raised suspicions of a problem with the water system. Results of water nitrate testing were negative, but total chlorine analysis yielded a high concentration of chloramines: 3.08 p.p.m. in the storage tank and 2.06 p.p.m. at the point-of-use stations. However, a negligible concentration of 0.02 p.p.m. was found in the tap water (Table 2). The concentration of free chlorine in the tap water was 0.33 p.p.m. (within the recommended range of up to 0.5 p.p.m.) [5] and, as expected, the levels in the storage tank and point-of-use stations were low.

Further investigation revealed that during the reconstruction and enlargement of the unit, the water system was disinfected by the maintenance company without notifying the clinical and technical staff of the hospital.

On consultation with water chemistry specialists, we learned that the iodide-DPD method is not specific for chlorine and chloramine and all oxidants capable of oxidizing iodide, including HP, react with iodide-DPD and are measured as total chlorine [6].

To correct the problem, additional flushings of the WTS were performed until no oxidant residue was detected by the DPD-iodide method at any of the point-of-use stations. No test for measurement of HP was available at our unit at the time.

The next dialysis session was performed uneventfully. Repeated blood tests for methaemoglobin showed normal levels in all children.

Discussion
Nitrates or chloramine presenting in the dialysate water have been implicated in methaemoglobinaemia and haemolytic anaemia in dialysis patients [1,7,8]. The investigations of Eaton et al. in 1973 [7] proved the direct relationship between the oxidative effect (severity of methaemoglobinaemia) and chloramine concentration in the water. The authors demonstrated that only carbon filter is effective in removing chloramines [7,8].

Nowadays, chloramines are still frequently used as a bactericidal agent to purify urban water. In most cases, the municipal supplier does not inform the dialysis unit staff about the time of chloramine addition, which poses a danger of overwhelming the carbon filter capacity and causing methaemoglobinaemia and haemolysis in the treated patients [9].

In our event, although the measurement of total chlorine seemed to indicate a high chloramine residue, it did not account for the appearance of air bubbles in the bloodlines. Moreover, even assuming complete failure of the carbon filters, the chloramine level in the storage tank cannot be higher than the level in the tap water. We also failed to observe any increase in conductivity of the reverse osmosis water due to deterioration of the reverse osmosis membranes, which would almost certainly have occurred had the chloramine concentration been high.

Since the disinfection of the water system with HP added directly to the tank was performed just before the

<table>
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<tr>
<th>Patient</th>
<th>Duration of dialysis (min)</th>
<th>Methaemoglobin (%)</th>
<th>Hb (g%)</th>
<th>ΔHb (%)</th>
<th>Haptoglobin (mg/dl)</th>
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<tr>
<td>1</td>
<td>120</td>
<td>11</td>
<td>6.7</td>
<td>5.6</td>
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</tr>
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<td>8.4</td>
<td>16.8</td>
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<td>4.2</td>
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</tr>
<tr>
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<td>3.4</td>
<td>10.8</td>
<td>14.8</td>
<td>74.2</td>
</tr>
<tr>
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<td>10</td>
<td>3.1</td>
<td>9.5</td>
<td>5.2</td>
<td>43.2</td>
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<tr>
<td>Mean ± SD</td>
<td></td>
<td>5.3 ± 2.4</td>
<td>9 ± 1.7</td>
<td>11.9 ± 5.9</td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.001.

Methaemoglobin, normal level < 1%; haptoglobin, normal level > 50 mg/dl.
event, and the oxidant was detected only in the tank and not in the tap water, it seemed reasonable to attribute the observed clinical phenomena to the presence of HP. Unfortunately, we could not directly measure HP because the dialysis unit was not equipped with the specific reagents at that time. (As the DPD-iodide method cannot be used to quantify HP, the measured values of 3.08 or 2.06 p.p.m. did not represent the actual concentration of peroxide in the dialysis water.) Our suspicions, however, were supported by the ample evidence of the effects of HP in the literature.

HP, an oxidative substance, is used extensively in medicine for its antiseptic qualities due to its ready release of oxygen. It is produced endogenously during electron transport processes in all aerobic cells and in the blood from auto-oxidation of haemoglobin [10].

Studies have shown that the in vitro exposure of normal human erythrocytes to increasing concentrations of HP is associated with methaemoglobin formation, lipid peroxidation and alterations in membrane function in a clear dose-dependent fashion [11]. This process is mediated by hydroxyl and ferrylhaemoglobin radicals created from interaction of HP with iron and other redox metals in a Fenton reaction [10,12]. Cellular defence mechanisms against the potential damage by HP consist of glutathione peroxidase and, especially, erythrocyte catalase. Catalase decomposes HP to water and oxygen without the generation of free radicals [13]. The amount of methaemoglobin generated on exposure of red cells to HP is inversely proportional to the cell catalase content [14].

The use of HP within enclosed body cavities or irrigation under pressure in surgical procedures has resulted in gas embolism and local emphysema due to the liberation of oxygen: 1 ml of 3% HP solution liberated 10 ml of O2 [15]. Colonic irrigation with HP, recommended in the past for meconium plug syndrome, was abandoned because of complications of gas embolism to the mesenteric and portal vessels, gangrenous bowel and subsequent bowel perforation [16]. Portal venous embolism was reported in a 2-year-old boy following ingestion of 3% HP solution [17]. The trial use of i.v. infusion of HP for extrapulmonary oxygenation in an animal study led to severe methaemoglobinemia [18]. I.v. administration of HP solution as unconventional therapy for AIDS resulted in severe acute haemolysis [19].

In our case, we speculate that the full activation of catalase on exposure to high concentrations of HP led to the formation of oxygen bubbles in the blood lines, but saturation of the finite amount of catalase left it incapable of preventing the oxidative effect and haemolysis.

The variability in severity of methaemoglobinemia in our patients is largely explained by the different duration of exposure to contaminated water (Table 2).

The presence of HP in the dialysis water was apparently caused by insufficient washing out of the system after disinfection. To the best of our knowledge, there is only one report of the exposure of dialysis patients to HP in the water [20]; this event was also recorded by CDC [21]. In this case, three children developed anaemia with an increased need for blood transfusions during 11 days following disinfection of the WTS with HP. As the test kit was not sufficiently sensitive to identify the scant remains of HP in the water, the washing of the system was inadequate.

A similar danger is posed by the peracetic acid–HP mixtures (Dialox, Puristeril), which are still being used to disinfect parts of dialysis systems [22].

This report highlights the critical need for stringent monitoring for disinfectants in haemodialysis to insure patient’s safety. Total chlorine analysis performed at the onset of each dialysis session is an efficient tool for determining the presence of oxidants in the dialysis water, provided the test is performed accurately. Special attention should be directed to the contact time between the reactants and the water sample (at least 3 min) [6] and the sensitivity of the method must be determined before testing. The method used must be adequate to measure low oxidant concentrations (0.1 p.p.m. total chlorine). If the results for total chlorine are positive, further analysis for HP residues must be performed with peroxide test strips [23]. This method is based on the oxygen transfer by peroxidase from the peroxide to an organic redox indicator, which is then converted to a blue-coloured product. The test is specific for HP and has a sensitivity of 0.5–25 p.p.m.

When changes in the WTS are being considered, all components of the system and their interaction should first be evaluated. The clinical staff should be well informed about the various breakdowns that can occur in the water system and their clinical repercussions. Ultimately, the responsibility for adequate water quality and the patients’ well-being is in the hands of the clinical staff.

Conflict of interest statement. None declared.

References
23. Merckoquant, Peroxide Test. Merck KGaA 64271, Darmstadt, Germany

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