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Research Article

CHANGES IN CONCENTRATION OF SODIUM HYPOCHLORITE OVER TIME: THE LABEL VERSUS REALITY

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A B S T R A C T

Household bleaches are the most common source of sodium hypochlorite used in root canal treatment. This study aimed to assess the concentration of free chlorine in 10 commercially available brands of household bleaches and its trend of change during four months. Ten different brands of commercially available household bleaches were analyzed to determine the concentration of free chlorine. For this purpose, titration of each product was performed four times at different intervals namely immediately after opening the lid and at one, three and four months following opening the lid. The initial concentration of all products was less than 5%, which was different from what it says on the label. At the end of four months, there was a mean reduction of 1.6% in the concentration of chlorine compared to its initial concentration. There was a significant decrease in chlorine concentration over time [P<0.001]

INTRODUCTION

The ultimate goal of root canal treatment is to eliminate debris and microorganisms from the root canals and shape them for efficient obturation in order to prevent apical periodontitis (MÖLLER, Fabricius *et al*. 1981). Considering the complex morphology of the root canal system, it is almost impossible to completely eliminate microorganisms from the root canals solely by mechanical preparation. Thus, mechanical instrumentation is often combined with irrigation of root canals to achieve this goal (Senia, Marshall *et al*. 1971, Rahimi, Janani *et al*. 2014). Sodium hypochlorite is the most commonly used irrigant for chemical debridement of the root canal system and is known as the gold standard due to its antimicrobial and tissue-dissolving properties (del Carpio-Perochena, Bramante *et al*. 2011, Mohammadi and Shalavi 2012).

As stated by Zehnder, sodium hypochlorite has most of the criteria required for an ideal irrigant (Zehnder 2006). It has broad-spectrum antimicrobial activity against microorganisms, even against hard to kill species such as enterococci and Actinomyces Actinomyces (Heling, Rotstein *et al*. 2001, Mahmoudpour, Rahimi *et al*. 2007, Nadalin, Perez *et al*. 2009). Moreover, in contrast to other irrigants, sodium hypochlorite is effective in both dissolving the remaining pulp tissues and removal of the organic components of the smear layer (Yamada, Armas *et al*. 1983, Baumgartner and Cuenin 1992). Low cost, availability and long shelf-life are among other favorable characteristics of sodium hypochlorite, making it a commonly acceptable irrigant (8).

There is no general consensus on the effective, and yet non-toxic, concentration of sodium hypochlorite in endodontics. A range of concentrations from 0.5 to 5.25% is commonly used by clinicians (Zehnder 2006). The minimum required concentration of sodium hypochlorite for tissue dissolution is 1% (Baumgartner and Cuenin 1992). Besides, the higher the concentration of sodium hypochlorite, the more the dissolved tissue (Mollashahi, Saberi *et al*. 2016) and the higher the expected cytotoxicity(Radcliffe, Potouridou *et al*. 2004). In order to have adequate antimicrobial activity, sodium hypochlorite must have a minimum concentration of 0.5% (14). However, a study showed that 5.25% concentration of sodium hypochlorite did not show a much higher antimicrobial activity than its 0.5% concentration (Byström and Sunvqvist 1985).

A study conducted in Australia showed that not only the use of sodium hypochlorite was more common among endodontists compared to general dentists but also endodontists tended to use sodium hypochlorite at higher concentrations (Clarkson, Podlich *et al*. 2003). Another study done through online questionnaires in the United States showed that the majority of dentists used sodium hypochlorite as the initial irrigant and 57% of them used minimum

concentration of 5% (Dutner, Mines *et al*. 2012). A study in Iran also revealed that 0.5% was the most common concentration of sodium hypochlorite used by dentists(Raoof, Zeini *et al*. 2015). Sodium Hypochlorite is an unstable and strong oxidizing agent (Collins, Allwood *et al*. 1981, WERE 1995). The concentration percentage of sodium hypochlorite in aqueous solutions compared to its weight shows the volume of the available free chlorine. Chlorine is available in two forms: HOCl and OCl(Mohammadi 2008). The level of available chlorine in sodium hypochlorite has a great impact on its activity (Johnson and Remeikis 1993, Rutala, Cole *et al*. 1998). The concentration of sodium hypochlorite depends on the initial concentration of chlorine in the purchased product, its storage conditions, rate of dilution, and the temperature at which it is used (Clarkson, Moule *et al*. 2001, Braitt, Rodrigues *et al*. 2013). Suzzete *et al*. investigated the concentration of the available chlorine in the solutions used by dentists in root canal treatment and showed that the measured concentration of chlorine was averagely 27% less than what was expected by the dentists. Moreover, the concentration of chlorine in 15% of the samples was less than the required concentration for disinfection and tissue dissolution (van der Waal, Connert *et al*. 2014). Time and environmental conditions are the main reasons, which could explain the difference between the real concentration and the expected concentration of sodium hypochlorite solution, especially when diluted.

Since household bleaches are the main source of hypochlorite used in endodontics, it would be helpful to know both the concentration of the available free chlorine in the purchased solution and its changes over time in dental office setting. Therefore, the aim of this study was to assess the changes in concentration of free chlorine in household bleaches available in the Iranian market over time.

MATERIALS AND METHODS

This experimental study was conducted on 10 different brands of commercially available household bleaches, which are the main source of sodium hypochlorite used by dentists in Iran (Table 1). Selected products had been produced in the past two months based on their production date. All samples were purchased from the same store in order to standardize the products in terms of their storage conditions.

Table 1 Brands of household bleaches evaluated in this study

1- Sehat bleaching liquid,5% , Sehat co., Iran
2- Rapido bleaching liquid,5% , Rapido co., Iran
3-Latifeh bleaching liquid,5%, Pak rokh co., Iran
4- Tage bleaching liquid,5.2%, Tage co., Iran
5- Ramooz bleaching liquid,5% , Ramooz co., Iran
6-Tirak bleaching liquid,5% , Tirak co., Iran
7-Golrang bleaching liquid,5%, Golrang co., Iran
8-Home plus bleaching liquid,5%, Iran
9- Active bleaching liquid,5%, Active co., Iran
10- Rakhsha bleaching liquid,5%, Paxan co., Iran

The first test was done immediately after opening the lid of the container for the first time. At this time, the containers' lid was examined to ensure absence of salt deposits around it; absence of salt deposits ensured no leakage through the lid. All sodium hypochlorite containers were then stored away from light and heat, similar to storage conditions in a standard dental clinic. During the experiment, all 10 containers of sodium hypochlorite were opened and shaken every morning six days a week in order to have a homogenous solution. All the trade labels were covered with a coded label in order to blind the experimenters. The following test was done in triplicate in order to detect changes in free chlorine level.

Chemical analysis

The first samples were acidified with acetic acid (Iran national standard number: 1994). Then each sample was titrated with 0.1 sodium thiosulfate ($Na_2S_2O_3$, Titripure; Merck, Darmstadt, Germany), until the solution became yellow. Next, 1mL of starch indicator solution (0.5 %) was added and titration was continued until the blue color disappeared. Each sample was tested three times and the average volume (mL) of the consumed sodium thiosulfate was calculated according to the formula below:

Free chlorine = V×N× 3.546

Where N is the normality of the standard solution of sodium thiosulfate, which equals 0.1 and V is the volume of the sodium thiosulfate solution in milliliters. This test was repeated in triplicate to determine the amount of free chlorine of the samples (27). The data were analyzed using SPSS software version 16.1 via descriptive statistics. One-way ANOVA was conducted to assess the change in concentration of chlorine over time.

RESULTS

The concentrations of free chlorine in different brands of household bleach at different time points during the four-month study period are shown in Table 2. Among the tested brands, only three had an initial concentration of higher than 5% and the rest had a concentration of less than 5%. As demonstrated in Table 2, free chlorine concentration in all brands of sodium hypochlorite decreased averagely by 1%, compared to the initial concentration at one month after opening the container lid. This reduction had a gradual trend during the next months, as at four months after opening the lid, an average decrease of 1.6% in free chlorine concentration was observed compared to the initial concentration (Figure 1).

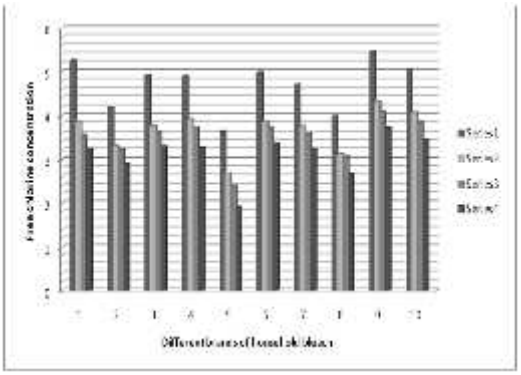

Figure 1 Changes in free chlorine concentration of different brands of household bleaches over time

There was a significant decrease in chlorine concentration over time [P<0.001; F(3.36)= 17.976].

Table 2 The measured concentration of free chlorine in different brands of household bleach during four months

Free chlorine concentration Brand code	2 months after production (Immediately after opening the lid)	3 months after production (1 month after first use)	4 months after production (2 months after first use)	6 months after production (4 months after first use)	Decrease in percentage of free chlorine after six months
1	5.26	3.85	3.56	3.23	39%
2	4.2	3.31	3.23	2.9	31%
3	4.92	3.78	3.63	3.3	33%
4	4.9	3.92	3.74	3.26	34%
5	3.65	2.69	2.41	1.91	48%
6	4.99	3.85	3.73	3.37	33%
7	4.72	3.76l	3.59	3.23	32%
8	3.99	3.13	3.07	2.66	33%
9	5.46	4.32	4.08	3.72	32%
10	5.07	4.07	3.85	3.44	32%
Mean (SD)	**4.72 (0.58)**	**3.67 (0.48)**	**3.49 (0.47)**	**3.1 (0.5)**	**34.7%**

DISCUSSION

This study indicated that four months after opening the lid of household bleach container, the average concentration of available free chlorine decreased to more than one-third of its initial concentration. This decrease in the amount of free chlorine had a sharper gradient during the first month of use compared to the next months.

Sodium hypochlorite used in endodontics is either household bleach or a dental product specifically made for this purpose; among which, the former is more commonly used by Iranian dentists. In this study, 10 different brands of commercially available household bleaches in Iran were evaluated. They all had a concentration of 5% except for one brand, which had a concentration of 5.2%. It takes several months for a product, from its production date to its distribution and retailing, to get into dental clinics for use. Therefore, the first test to determine the level of available free chlorine was done two months after the date of production, which corresponds to the time when consumers usually purchase these products.

The first titration test done on the 10 different products showed that only three brands had an initial concentration higher than 5%, while the initial concentration of two brands was less than 4%. The mean initial concentration of 4.7% in the products was consistent with the concentration labeled on the container (three brands even had a concentration more than what was labeled). The initial non-diluted concentration, however, was generally lower than the recommended concentration of 5.25%, which is often used in endodontics (Banchs and Trope 2004). Eight brands had a concentration less than 4% at one month after the first use of the solution, and the concentration of those three products that had a concentration of 5% and more in the first test, decreased to less than 5%. The mean concentration of the products was almost 3.5% and 3.1% in the third and fourth tests, respectively, which were done two and four months after the use of the solution (four and six months after the production date), respectively.

There is still some debate regarding the most efficient concentration of sodium hypochlorite for use in endodontics. A broad range of concentrations between 0.5-5.25% is recommended while this range was extended to 10% in a previous study (Matsumoto, Nagai *et al*. 1987).

Some studies have shown that higher concentrations of sodium hypochlorite are more effective against Enterococcus faecalis and Candida albicans (Waltimo, Ørstavik *et al*. 1999, Gomes, Ferraz *et al*. 2001). Moreover, higher concentrations of sodium hypochlorite have greater capability for tissue dissolution (Hand, Smith *et al*. 1978). Nonetheless, lower concentrations in some studies were shown to have the same effect as higher concentrations (Moorer and Wesselink 1982, Siqueira, Rôças *et al*. 2000). However, severe irritation following accidental extrusion of 5.25% sodium hypochlorite into the periapical tissue (Hülsmann and Hahn 2000), negative effects of higher concentrations on the modulus of elasticity (Sim, Knowles *et al*. 2001) and time-dependent cytotoxicity of higher concentrations against stem cells of the apical papilla in regenerative endodontic procedures have also been reported(Mollashahi, Saberi *et al*. 2016). In spite of these findings, it seems that the concentration of sodium hypochlorite is actually a dentist's choice.

Our study is similar to that of van der Wall (van der Waal, van Dusseldorp *et al*. 2014), in that both showed that household bleaches had a concentration less than 5% and if diluted, a significant difference between the expected concentration and the real concentration of free chlorine would occur. However, if we accept the existing evidence-based agreement that 1% concentration of sodium hypochlorite has sufficient clinical efficacy, almost all the products examined during four months at a dilution ratio of 1:1 have optimal concentration for use in root canal treatment. Another interesting finding of our study was the change in level of free chlorine during four months. The average decrease in free chlorine concentration was 22.3%, 5% and 11.5% during the first, second and the next two months of the study, respectively. The results showed that the average concentration of free chlorine decreased from 4.7% to 3.1% during four months of the study, which was equal to 34.7% decrease compared to the initial concentration. These results show that, in clinical use of these materials, the reduction in the amount of free chlorine is the greatest during the first month of use, and this reduction continues at a lower pace in the next months.

Sodium hypochlorite is a very unstable solution and the concentration of its free chlorine depends on the pH, time(Carlotto, Luisi *et al*. 2016), temperature, exposure to light, contact with air and presence of metal ions (Clarkson, Moule *et al*. 2001). In this study, the products were stored at

room temperature (25˚C), in normal light and in opaque plastic containers that were air-tight. To simulate the normal storage conditions in dental office setting, the containers were opened every day. Based on the above explanations, a decrease of nearly 35% in the concentration of free chlorine may not be in line with the study of Farr *et al*, who concluded that solutions with concentrations less than 6% and pH of 11 and higher have acceptable shelf-life in temperatures below 30˚C. However, there is no agreement regarding the definition of "an acceptable shelf-life". One of the limitations of our study was that pH was not studied. The present study is also consistent (to some extent) with anotherstudy (Pişkin and Türkün 1995), which demonstrated that solutions containing free chlorine experienced a rapid decrease in chlorine concentration during a period of 200 days. However, in their study, a relative stability in chlorine concentration was noted during the first month followed by a sharp decrease in the next months while our study showed a sharp decrease in the first month followed by a slower trend of reduction in the next months. It is not well clear for the authors why such a sharp decrease occurred in the first month; however, it may be due to chemical reactions in the bottle and also thermal variations during transfer of products from Tehran (where the manufacturing companies are located) to Zahedan city (about 1500km far from Tehran) with totally different weather conditions. During the transfer, gases may be produced in the containers, which would be released immediately after opening the lid. Our study was not consistent with that of Papplardo *et al*, either (Pappalardo, Tanner *et al*. 1985), since they demonstrated a decrease of 70% in the concentration of free chlorine.

In absence of sodium hypochlorite irrigating solutions specifically made for endodontic purposes, any estimate regarding the used concentration is imprecise and as concluded by van der Wall *et al*, the exact concentration of free chlorine in household bleaches cannot be known without analysis (van der Waal, van Dusseldorp *et al*. 2014).

Further studies are required to assess the decrease in the concentration of these solutions during longer periods of time and also under different storage conditions. Assessment of the change in their pH is also an interesting topic for future studies.

CONCLUSION

This study showed that the concentration of free chlorine was averagely less than 5%, immediately after opening the container lid. This rate decreased to one-third after four months of use. The difference between the real concentration and the expected concentration should be taken into account when diluting these solutions.

The use of low-volume disposable packages for sodium hypochlorite, sufficient for use in just one patient, may be a solution to ensure that the required concentration of sodium hypochlorite is being used in endodontics.

Competing interests

We declare that the authors have no competing interests.

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