Plasma Level Formaldehyde in Children Receiving Pulpotomy Treatment under General Anesthesia

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Objectives: Formocresol has long been used by dentists for pulpotomy of primary teeth. Due to some concerns regarding its possible carcinogenicity, formocresol has been the topic of numerous studies. This study sought to assess the changes in plasma level of formaldehyde of children after receiving pulpotomy under general anesthesia. Study design: Twenty-five children between 2-6 years requiring dental treatments under general anesthesia were studied. Blood samples were taken of children before and after the procedure. Plasma level of formaldehyde was measured using high performance liquid chromatography (HPLC). Results: A total of 106 pulpotomy treatments were performed in 25 children using 126 cotton pellets dipped in formocresol. An increase and a decrease in plasma level of formaldehyde were noted in 5 (20%) and 20 (80%) children, respectively post-operatively compared to baseline. The t-test showed no significant difference in plasma level of formaldehyde pre- and postoperatively (P=0.12). the plasma level of formaldehyde in children who had higher levels of formaldehyde prior to the operation was also higher than that of others after the operation and this association was statistically significant (P=0.001, r=0.64). Conclusions: The results showed no significant change in the mean plasma level of formaldehyde in children who received pulpotomy under general anesthesia compared to its baseline value.

Key words: Formaldehyde; Pulpotomy; Primary teeth; Chromatography

INTRODUCTION

Formocresol is used for pulpotomy treatment only in primary teeth with high clinical and radiographic success rate; thus, it is considered as the gold standard of pulpotomy treatment and is the most commonly used material for this purpose [1]. The Buckley's formocresol contains 19% formaldehyde, 35% cresol and 17.5% glycerin. It must be kept at 15-30°C and freezing must be avoided [2]. The history of using formaldehyde-containing medications for pulpotomy dates back to 1874 when Nitzel used tricresol formalin for this purpose [3]. The Buckley's formocresol was produced in 1904 [4]. At present, some concerns exist regarding the adverse effects of formocresol due to the presence of formaldehyde in its formulation. Studies have shown that formaldehyde present in formocresol is systemically absorbed [5,6]. A study on dogs showed that 10% of formaldehyde present in formocresol was systemically absorbed [5]. Another study on rats revealed that labeled formaldehyde, used for the pulpotomy of a molar tooth, was later found in some body organs of rats [6].

Generally, formaldehyde enters into the human body via the air, water and foods. According to the World Health Organization, formaldehyde intake is 1.5-14 mg/day (mean value of 7.8mg) from foods, 0.2mg/day from drinking water and approximately 1mg/day from respiration; it means that an adult receives 9mg/day formaldehyde from the outside environment [7,8]. However, the amount of formaldehyde intake is variable in different geographical locations

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[9]; this rate has reported to be 11mg/day in the North America [9]. Children receive less formaldehyde due to less consumption of foods; however, an exact value has not been reported [10]. In 0.5-1ppm concentration, it is hazardous for health especially when inhaled or contacting the eyes [11]. The Health and Safety Executive (HSE) (2002) has reported the occupational exposure limit for formaldehyde to be 2ppm in 8 hours for a worker [12].

Human body metabolizes exogenous and endogenous formaldehydes [13-15]. Averagely, 3-12 ng/g formaldehyde is synthesized endogenously [16]. The normal serum level of formaldehyde is 0.0127 to 2.28 µg/mL or ppm in humans [17].

Exogenous formaldehyde is absorbed via nutrition, respiration or skin contact. However, the amount of formaldehyde absorption via skin contact is scarce. Formaldehyde in the diet is rapidly absorbed by the gastrointestinal tract and carries a risk of intoxication [18]. Inhaled formaldehyde is rapidly absorbed by the upper respiratory system and metabolized; a study on monkeys and rats showed that after inhalation of formaldehyde, its serum level did not increase [19].

Formaldehyde is metabolized in the human body and releases a carbon atom. These free carbon atoms create a one-carbon pool and are used for biosynthesis of purine, thymidine and some other amino acids. Thus, formaldehyde affects the structure of RNA, DNA and protein [20].

The main concerns with regard to the use of formocresol include immune sensitization and carcinogenicity, genotoxicity and mutagenicity of formaldehyde in its formulation. The genotoxic potential of formaldehyde has been reported in studies on culture media in mammals [13,21]. Antibody formation against formaldehyde after pulpotomy treatment with formocresol has been reported in dogs and indicates immune sensitization [22]. Moreover, formaldehyde covalently bonds to the amino and sulfhydryl groups of DNA and forms DNA-protein cross link (DPX), which is an unstable hydroxymethyl protein [23-26]. Unrepaired DPX can stop the process of DNA replication and exert a genotoxic effect known as sister chromatid exchange (SCEs)[27,28].

It is ranked as a group 1 carcinogenic agent by the International Agency for Research on Cancer (IARC)[29]. It is also categorized as a carcinogenic material by the Agency for Toxic Substances and Disease Registry and Health Canada Disease Registry [30]. Thus, protective and safety measures must be taken when working with formaldehyde.

The toxic effects of formocresol due to its formaldehyde content cannot be denied. Formaldehyde is synthesized in the human body and also enters into the body exogenously [10]. It is then metabolized in the body [31]. Considering the systemic absorption of formaldehyde used for pulpotomy treatment [5,6], a question arises that how much of the formaldehyde applied enters the blood circulation? And what would be the outcome of absorbed formaldehyde? Would it be totally metabolized or there is a risk of bonding to DNA and exerting its mutagenic effects? Considering the excellent clinical results of formocresol and the existing concerns regarding its safety, this study sought to assess the changes in plasma level of formaldehyde of children after receiving pulpotomy under general anesthesia since formocresol pulpotomy of several tooth within one session may increase the risk of toxicity.

MATERIALS AND METHOD

This study was conducted on 25 children between 2-6 years with the ethical approval of Shahid Beheshti University of Medical Sciences, ethics committee. The children were examined by a pediatric dentist and those requiring pulpotomy treatment upon their parents' request for treatment under general anesthesia (due to their poor cooperation) were chosen for this study. The study was performed in a hospital in Tehran. Written informed consent was obtained of all parents or legal guardians of children. Patients were examined by an anesthesiologist for any systemic condition. Children were requested to refrain from eating solid foods for 8 hours and drinking for 2 hours prior to general anesthesia.

All pulpotomy treatments were performed by the standard method using the Buckley's formocresol (Sultan, USA). Standard pulpotomy treatment was performed; all caries were removed and access cavity was prepared. The entire pulp chamber roof was removed and the pulp chamber was rinsed with mild flow of water injected by a syringe. Pulp chamber was dried with cotton pellets. Cotton pellets were then dipped in Buckley's formocresol and pressed by gauze to remove excess formocresol. The cotton pellet was then placed in the pulp chamber in such a way that it contacted the canal orifices. It remained at the site for 5 minutes and was then removed. A thick paste of zinc oxide eugenol was then applied on the pulp tissue at the orifices.

Blood sampling

Two blood samples were collected of children. A preoperative blood sample was taken as a reference for later comparison and another sample was taken postoperatively. Two milliliters of peripheral blood were taken preoperatively and two milliliters was taken postoperatively (totally 4cc). The first blood sample was drawn via the venous catheter prior to the administration of anesthetic agent and Ringer's solution. The second blood sample was taken after discontinuation of Ringer's solution and termination of operation via the venous catheter by the anesthesiologist. The samples were sent to the Pharmacognosy Research Center of Shahid Beheshti University.

High performance liquid chromatography (HPLC)

The HPLC system (Knauer, Berlin, Germany) with a UV detector, 1001-K pump (Knauer, Berlin, Germany), 2800-K detector (Knauer, Berlin, Germany), 7525 injection valve, 20µL sample loop and ChromGate® data processing software were used. The Knauer C18 column (150 mm x 4.6 mm) was used and the mobile phase included acetonitrile -water (55:45 isocratic) at a flow rate of 1 mL/min and detection was at 350nm maximum wavelength.

Plasma samples

A total of 10µL of 5N phosphoric acid and 100 µL of 1mg/mL 2,4 dinitrophenylhydrazine were added to 500 µL of plasma sample; the mixture was stirred for one hour. For lower volumes of plasma samples, 2,4 dinitrophenylhydrazine and phosphoric acid were used in proportionately lower volumes. After derivatization, plasma samples were kept frozen until injection to HPLC [31]. To determine the plasma level of formaldehyde, first 1, 0.5, 0.25, 0.125 and 0.0625ppm concentrations of formaldehyde were injected to the system to draw a calibration curve.Based on the drawn curve and the obtained formula (where x is the concentration and y is the area

under the peak), level of formaldehyde in the plasma samples was measured. For each sample, a curve was drawn by the system and the area under the peak was measured using the formula derived from the pure formaldehyde. The plasma concentration of formaldehyde was calculated as such (Figure 1).

Statistical analysis

The data analysis was performed by IBM SPSS 20.0.1 (IBM Corp., Armonk, NY, USA). ANCOVA was used to assess the effect of age, sex and number of cotton pellets used on plasma level of formaldehyde after anesthesia. The level of formaldehyde in the plasma samples before and after procedure were analyzed by paired t-test.

RESULTS

Twenty-five children were evaluated in this study including 18 males (72%) and 7 females (28%). Fifty blood samples were collected (25 preoperative and 25 postoperative samples). A total of 106 teeth were pulpotomized .

There were 9 children 2 years of age (36%), 9 children 3 years of age (36%), 4 children 4 years of age (16%), 2 children 5 years of age (8%) and one child 6 years of age (4%). The mean age of children was 2.08 years with a standard deviation of 1.12 years. Table 1 shows a list of pulpotomized teeth.

The mean concentration of formaldehyde in preoperative plasma samples was 0.34 ppm with a standard deviation of 0.15, a maximum of 0.58ppm and a minimum of 0.03ppm.

The mean concentration of formaldehyde in postoperative plasma samples was 0.26ppm with a standard deviation of 0.24, a maximum of 1.45ppm and a minimum of 0.11ppm.

The mean change in the concentration of formaldehyde in plasma samples was -0.9ppm with a standard deviation of 0.27, a maximum of 1.06ppm and a minimum of -0.32ppm (Table 2). The t-test showed no significant difference in plasma level of formaldehyde pre- and postoperatively (P=0.12).

The Spearman's rho showed an association between formaldehyde values before and after anesthesia; the plasma level of formaldehyde in children who had higher levels of formaldehyde prior to the operation was also higher than that of others after the operation and this association was statistically significant (P=0.001, r=0.64).

In general, a reduction and an increase were noted in plasma formaldehyde levels of 20 (80%) and 5 (20%) children, respectively after the operation compared to preoperative values; the mean

Table 1. Number of patients, pulpotomized teeth

	Males	Females
Number of patients (percentage)	18 (72%)	7 (28%)
Number of pulpotomized teeth	76 (71.7%)	30 (28.3%)

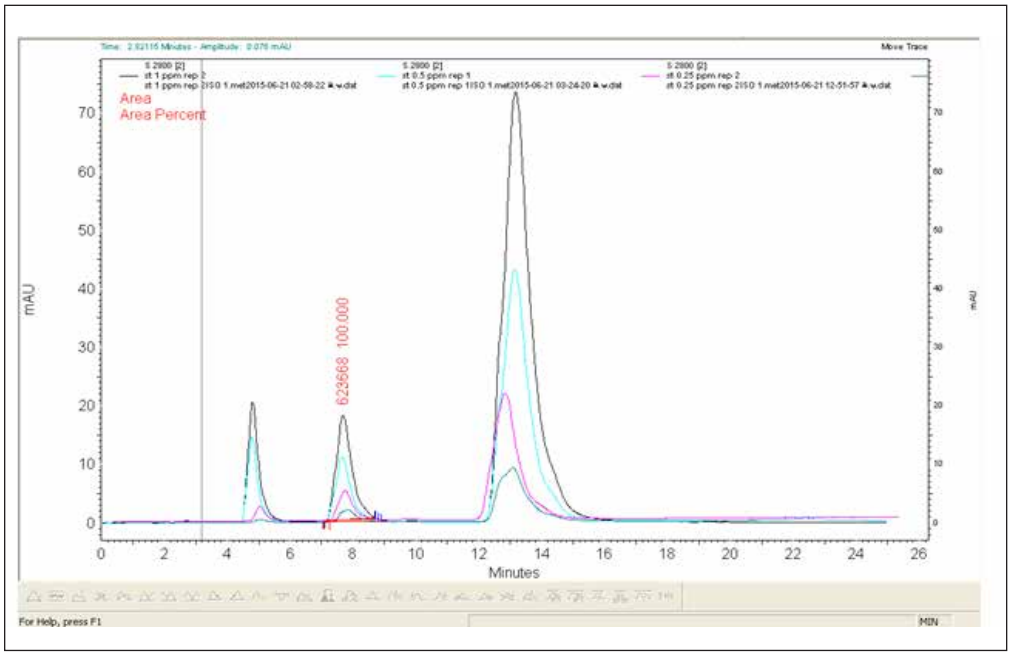
Table 2. Pulpotomized teeth

Tooth	Right	Left	Total
Upper A	10 (9.3%)	7 (6.7%)	17 (16%)
Upper B	8 (7.5%)	7 (6.7%)	15 (14.2%)
Upper D	12 (11.3%)	11 (10.4%)	23 (21.7%)
Lower D	16 (15%)	13 (12.4%)	29 (27.4%)
Upper E	1 (0.95%)	5 (4.85%)	6 (5.7%)
Lower E	8 (7.5%)	8 (7.5%)	16 (15%)

Table 3. Changes in plasma level of formaldehyde

Plasma level of formaldehyde (ppm)	Mean	Standard deviation	Maximum	Minimum
Before	0.34	0.15	0.58	0.03
After	0.26	0.24	1.45	0.11
Change	-0.9	0.27	1.06	-0.32

Figure 1. Measuring the plasma level of formaldehyde by HPLC



change in concentration of formaldehyde was -0.9ppm with 0.27 standard deviation, a maximum of 1.06ppm and a minimum of -0.32ppm.

No significant correlation was found between the number of cotton pellets used and change in plasma level of formaldehyde (P=0.49, S=-0.14). ANCOVA was used to assess the effect of age, sex and number of cotton pellets used on plasma level of formaldehyde after anesthesia. The results of ANCOVA showed that age (P=0.87), sex (P=0.54) and number of cotton pellets (P=0.74) had no significant effect on plasma level of formaldehyde.

DISCUSSION

Formocresol is used for pulpotomy treatment with high clinical and radiographic success rate; thus, it is considered as the gold standard of pulpotomy treatment and is the most commonly used material for this purpose [1]. At present, some concerns exist regarding the adverse effects of formocresol due to the presence of formaldehyde in its formulation. Despite several studies on toxicity, carcinogenicity and mutagenicity of formocresol, it is still widely used. Yoon et al. questioned 92 pediatric dentists regarding their material of choice for pulpotomy treatment. The results showed that 61% reported using formocresol; 28% reported using full strength and 33% reported the use of diluted version [32].

In the current study, blood samples were collected pre and postoperatively of 25 children between 2-6 years requiring dental treatment under general anesthesia to assess the plasma level of formaldehyde and its possible change. Number of cotton pellets used and its correlation with plasma level of formocresol was also assessed. Blood samples were sent to a laboratory and subjected to HPLC; reduction and increase in plasma level of formaldehyde were noted in 80% and 20% of patients postoperatively. No significant association was found between plasma level of formaldehyde and number of cotton pellets used (P=0.49). It should be noted that the reported decrease (which may not be expected) and increase in serum level of formaldehyde were small and statistically insignificant (P=0.12). Several factors may explain this slight decrease in plasma level of formaldehyde postoperatively. It should be noted that the children were refrained from eating and drinking for 8 and 2 hours prior to the operation, respectively and this process continued during anesthesia as well. However, formaldehyde in the blood stream is absorbed and metabolized by cells and tissues; thus, irrespective of the use of formocresol, reduction in plasma level of formaldehyde is expected during this period. Moreover, under general anesthesia, ventilation is provided via an oxygen mask and the children do not breathe ambient air, which often contains formaldehyde. Thus, during anesthesia, the three exogenous sources of formaldehyde, i.e. water, air and food [8] were not present and only formocresol, containing formaldehyde, was used. We noticed changes with regard to plasma levels of formaldehyde, which were all statistically insignificant. Also, children received 100cc of Ringer's solution during the operation; although this amount may be insignificant for an adult, it may affect the plasma concentration of materials in children since they have 2-3L of blood. Considering all the above, the reduction in plasma level of formaldehyde is justified. The formaldehyde in formocresol is probably used for tissue fixation and only a small amount may enter into the blood circulation.

Heck et al, [19] and Casanoa-Scmitz et al [33] assessed the concentration of formaldehyde in human blood, Fisher-344 rats and monkeys after inhalation of formaldehyde.

Heck et al [19] evaluated the concentration of formaldehyde in human blood and Fisher-344 rats after inhalation of formaldehyde under controlled conditions. They exposed 8 rats to 14.4ppm formaldehyde for 2 hours and 6 humans to 1.9 ppm formaldehyde for 40 minutes. Blood samples were taken before and after the experiment, and concentration of formaldehyde in the blood of humans and rats was measured by GC-Mass chromatography. The changes in formaldehyde concentrations were not significant. The results were variable in humans since some showed increase and some others showed decrease in formaldehyde concentration in blood. Their findings were in line with ours. Casanoa-Scmitz et al [33] evaluated the blood level of formaldehyde in monkeys after formaldehyde inhalation. They exposed six young monkeys to 6ppm formaldehyde for 5 days a week for 4 weeks and each time for 6 hours; blood levels of formaldehyde were measured using GC-Mass chromatography; the results showed no significant difference in blood level of formaldehyde, which was similar to the results of Heck et al. However, assessment of nasal tissue in another study revealed that in concentrations over 2ppm, formaldehyde exerted genotoxic effects [34]. In other words, although the blood level of formaldehyde did not change significantly after exposure to 14.4ppm [19] and 6ppm [33] formaldehyde, 2ppm formaldehyde was found to be mutagenic. However, Kahl et al [35] reported that formocresol used for pulpotomy is safe due to its small dose. They assessed 30 children between 2-6 years under general anesthesia and measured the blood level of formaldehyde using GC-Mass chromatography. However, they could not determine its concentration in blood and only detected its presence. On the contrary, Luo et al, in 2001 introduced HPLC along with fluorescence for measurement of blood level of formaldehyde [36]. Wang Yong-Sheng et al. used HPLC and reported the normal blood level of formaldehyde to be between 0.0127-2.28µg/mL or ppm [17]. In the current study, HPLC was used to measure the plasma level of formaldehyde. Based on all the above, it can be concluded that use of the same amount of formaldehyde may result in variable blood levels in different individuals. Zarzar et al [37] evaluated the mutagenicity of formocresol following pulpotomy of primary teeth and showed that it had no significant mutagenicity in 29 children who received formocresol pulpotomy. However, use of full strength formocresol was severely mutagenic for one patient; this result was concerning. To assess the toxicity of formocresol, cell culture tests must be carried out as performed by Zarzar et al [37] and Da Silva et al [38] and increase or decrease in blood level of formaldehyde is not sufficient to rule out the toxicity of formocresol used for pulpotomy.

Milnes stated that antibiotics are extensively used and have caused death of human beings so why we should be concerned about the use of formocresol [39]. However, we should cut down the use of drugs with potentially adverse effects especially when safer alternatives are available.

Concerns regarding the use of formocresol in pediatric dentistry is due to the presence of un-metabolized formaldehyde, which enters into the blood circulation, reacts with macromolecules and exerts mutagenic effects on the liver, kidneys, muscles, heart, spleen and the lungs [5,40,41].

Several studies have compared the toxicity of formocresol and that of other formalin-containing compounds. Gahyva et al [42] evaluated 14 chemicals used in root canal treatment of teeth in terms of genotoxicity and mutagenicity using two prokaryotic tests namely SOS chromotest and Ames test; among the tested compounds, formocresol was the only compound with severe genotoxic effects.

Kabaktchieva et al [43] compared the cytotoxicity of drugs used for pulp treatment of primary teeth. They used MTT dye reduction assay and ELISA to assess the cytotoxicity of mineral trioxide aggregate (MTA), calcium hydroxide and Resorcinol solution in formalin (RF) and reported that MTA and calcium hydroxide were safer than RF for pulpotomy.

All the above-mentioned studies have been conducted in vitro and cells were directly exposed to formaldehyde in the composition of formocresol, which is different from the in vivo application of formocresol, and questions regarding the toxicity of formocresol remain. The genotoxic potential of formaldehyde has been confirmed in cell culture studies in mammals [44,45]. Zarzar et al [37] assessed the mutagenicity of formocresol following its use for pulpotomy of primary teeth and reported no significant mutagenic effect except in one patient. Due to mutagenicity of formocresol in one child, they raised concerns regarding the use of formocresol. Da Silva et al [38] evaluated DNA damage of human lymphocytes due to paramonochlorophenol, calcium hydroxide and formocresol at 100 μg/mL concentration using single cell gel assay (comet) technique, which is fast and fluorescent-sensitive for detection of DNA damage. They concluded that these three materials at 100 μg/mL concentration do not cause DNA damage in peripheral lymphocytes and reported that low concentration of formocresol was safe for pulpotomy. Leiste et al [15] evaluated the genotoxic effects of formocresol on lymphocyte culture using the Moorhead's method. The samples were evaluated for chromatid gap, isochromatid gap, chromatid break, isochromatid break, other chromosomal alterations and total alterations. Their results were in contrast to those of Zarzar et al, although they both used formocresol in the same approach. For this reason, they recommended that working with formocresol must be done with caution.

Formaldehyde is a reactive, water-soluble gas, rapidly absorbed by the upper respiratory system in high concentrations [11]. It can cause nasal tumor in animals and probably nasopharyngeal cancer in humans [46]. The IARC ranked formaldehyde as a group one carcinogen based on a report on increased mortality rate due to nasopharyngeal carcinoma among industrial workers in the United States by the NCI and a systematic review of the literature [47]. Moreover, the Agency for Toxic Substances and Disease Registry and the Health-Canada-Disease Registry classified formaldehyde as a carcinogen [30]. Since formaldehyde was shown to be carcinogenic in animal models, it cannot be tested on humans. Researchers in the Chemical Industry Institute of Toxicology (CIIT) Center for Health Research designed a three-dimensional dynamic model to simulate the nasal air flow and transport of formaldehyde gas and formaldehyde deposited on the mucosal surfaces of rodents, monkeys and humans [48]. They reported that rate of cancer following contact with formaldehyde is negligible unless its concentration reaches 600-1000 ppb [49].

Several studies have searched for alternatives for formocresol. Markovic et al [50] evaluated formocresol, calcium hydroxide and ferric sulfate in terms of clinical and radiographic success and concluded that all three had acceptable results; ferric sulfate yielded results comparable or even superior to those of formocresol.

Concerns with regard to the use of formocresol led to numerous investigations; however, no consensus has been reached in this respect. In our study, no significant change occurred in plasma level of formaldehyde after the operation; however, even insignificant amounts may be safe in a child but cause problems in another. Patients with systemic conditions or immunocompromised children may be at higher risk. Also, this gas is rapidly absorbed by the upper respiratory system and considering the risk of nasopharyngeal carcinoma associated with its inhalation by the clinicians and office staff, use of safer alternatives such as calcium hydroxide, glutaraldehyde, zinc oxide eugenol, paraformaldehyde, electro surgery, MTA Ledermix, KRI paste, ferric sulfate, laser and bioceramics is recommended [51-55].

CONCLUSIONS

The results of the current study showed no significant change in plasma level of formaldehyde after conduction of several pulpotomy treatments under general anesthesia. Number of formocresol-dipped cotton pellets used had no significant association with change in plasma level of formaldehyde.

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