Blood Gas Tension and Acidity Level of Caries Exposed Vital Pulps in Primary Molars

Aviv Shmueli*/ Marcio Guelmann **/ Nili Tickotsky ***/Rinat Ninio-Harush ****/
Avia Fux Noy ***** / Moti Moskovitz ******

Objective: *The purpose of this study was to determine if gas tension and acidity levels could serve as biomarkers of pulp inflammatory status in primary dentition.* ***Study design:*** *The present study evaluated acidity level and blood partial pressures of O_2 and CO_2 collected from vital pulp chambers of 84 primary molars with deep carious lesions encroaching the pulp. Teeth were treated with pulpotomy or pulpectomy based on clinical judgement. Pulpectomy was performed when symptoms of spontaneous pain, difficulty in obtaining hemostasis and/or dark purple blood were present. Using a glass capillary, pulp chamber bleeding was collected and within ten minutes a neonate Astrup test was performed to determine blood gas module pH, pCO_2, and pO_2.* ***Results:*** *Eighty-four children with one affected tooth participated in the study (37 girls and 47 boys). Age ranged between 3.5 to 9-years (average: 5.3 years). Seventy-one (84%) were treated with the aid of inhalation analgesia, conscious sedation or general anesthesia. Pulpotomy was performed on 58 teeth (69%). Teeth undergoing pulpectomy revealed significant higher level of CO_2 partial pressure ($P= 0.002$). Acidity level values (pH) were also lower but none significantly in teeth with pulpectomy ($P= 0.137$).*
Conclusions: *Higher pCO_2 levels was found in pulps needing pulpectomy.*

Keywords: *Blood gas monitoring, Acidity levels, Biomarkers, Primary dentition.*

INTRODUCTION

Vital pulp therapy in primary teeth is indicated for extensive tooth decay approximating the pulp of asymptomatic teeth when caries removal results in a carious or mechanical pulp exposure or teeth with signs of reversible pulpitis where the remaining radicular tissue is judged to be vital without suppuration, purulence, necrosis, or excessive hemorrhage that cannot be controlled by a damp cotton pellet after several minutes.[1] Pre-operative diagnostic methods of pulp status in primary teeth are primarily based on clinical signs and symptoms, pain history and radiographic assessment.[2-4]

After carious pulp exposure in vital primary teeth, treatment options are pulpotomy[5,6] or pulpectomy.[1] The success of vital pulp therapy strongly depends on the inflammatory status of the coronal and radicular pulp tissues.[7] While for healthy pulp tissue or with inflammation confined to the coronal pulp, the recommended treatment is pulpotomy, for irreversible pulpitis the recommended treatment is pulpectomy.[8] Clinicians face the diagnostic difficulty of accurately assessing the inflammatory state of the pulp,[3] a diagnosis that will determine tooth prognosis.[3] Ricucci [3] found in permanent teeth that cases with normal pulp and reversible pulpitis had higher correlation between the clinical and histologic diagnosis of pulp conditions than cases of irreversible pulpitis (96.6% and 84.4%, respectively). Endodontic diagnostics showed higher competence at identifying persons who were free of pulpitis than at identifying pulpitis-positive persons, with a danger of false-positive misclassifications.[9]

*Aviv Shmueli, DMD, Department of Pediatric Dentistry, Hebrew University Hadassah School of Dental Medicine, Jerusalem, Israel.
**Marcio Guelmann, DDS, Department of Pediatric Dentistry, University of Florida College of Dentistry, Gainesville, USA.
***Nili Tickotsky, DMD, MSc, Med, Department of Immunology, Weizmann Institute of Science, Rehovot, Israel.
****Rinat Ninio-Harush DMD, Department of Pediatric Dentistry, Hebrew University Hadassah School of Dental Medicine, Jerusalem, Israel.
*****Avia Fux Noy, DMD. Department of Pediatric Dentistry, Hebrew University Hadassah School of Dental Medicine, Jerusalem, Israel.
******Moti Moskovitz, DMD, PhD, Department of Pediatric Dentistry, Hebrew University Hadassah School of Dental Medicine, Jerusalem, Israel.

Send all correspondence to:
Aviv Shmueli, Department of Paediatric Dentistry, Hadassah School of Dental Medicine, P.O. Box 12272, Jerusalem 9112102, Israel.
Phone: 972-2-6776122
Fax: 972-2-6431747
E-mail: aviv.dentist@gmail.com

In the dental pulp, being an environment of low compliance and limited access constricted by the surrounding hard tissues, inflammation may induce unrepairable cell damage. In the case of pulpitis, inflammation increases the damage created by the bacterial infection.[10,11] Hypoxia influences the inflamed tissue environment by regulating oxygen-dependent gene expression.[12] Current next-generation diagnostics, designed to assess the inflammatory state of the pulp of permanent teeth at chairside, use blood or other tissue fluid to find quantitative biomarkers of disease,[13,14] such as TGF- β,[15] nitric oxide synthase[16] and histological composition of inflammatory cells in the inflamed pulp.[17] So far, many inflammation-related factors have been identified,[18,19] but no reliable, plain biomarkers that clearly distinguish irreversible pulpitis from reversible inflammation have been found.[4]

Operative diagnosis currently relies on the color of exposed pulpal blood, bleeding duration and hemostasis achievement,[21,22,] as these parameters do not necessitate any advanced technologies. Mutluay *et al* evaluated bleeding control at the exposure site and at orifice site of primary molars with caries exposures. They concluded that hemostasis at the orifice site is paramount for the success of pulpotomy procedures.[21] Aaminabadi *et al* assessed the relationship between pulp blood color and the histological and hematological features of the pulp tissue during pulpotomy and pulpectomy in primary molars. They found blood color significantly darker in the pulpectomy group and attributed it to the existence of more inflammation, stasis of blood in pulp tissue, decrease of pH, increase of temperature, and poorly oxygenated hemoglobin.[20] The problem with blood color is that it is challenging to quantify it and thus, it cannot serve as a biomarker. In the present study, we aimed to quantify some of the factors that influence blood color and check if they can serve as biomarkers. Respiratory measurements such as oxygen probe and CO_2 release techniques have been suggested to yield more sensitive indicators of pulp tissue injury than previously used histological techniques.[23]

Blood is normally slightly basic, alkaline, with a pH range of 7.35 to 7.45.[24] The pH of the extra cellular fluid in normal conditions is 7.4. Acidosis has been reported in inflamed tissues, and ions changes at the site of inflammation may provoke alterations in blood acid-base status.[25,26] Dental pulp is exposed to changes in extracellular pH under exposure to caries-associated bacteria.[27] Acidic extracellular pH condition is associated with growth arrest or death of dental pulp stem cells.[27] Oxygen saturation levels are an effective indicator of pulp vitality in primary teeth[28,29] as hypoxia promotes inflammation[12] and vice versa, leukocytes decrease pulp tissue O_2.

The present study evaluated the level of acidity and blood partial pressure of O_2 and CO_2 in samples drawn from pulp chambers of primary molars undergoing pulpotomy or pulpectomy. Our aimed to examine whether differences exist in pH, pCO_2 and pO_2 between reversible and irreversible pulpitis that may serve as a basis for the development of a chair-side diagnostic technique. We hypothesized that these parameters may differ significantly between the two states of pulp status.

MATERIALS AND METHOD

Study Population

Healthy children, three to nine years old, undergoing scheduled dental treatment at the Department of Pediatric Dentistry were recruited for the study. The inclusion criteria for the pulpotomy group consisted of primary molars with deep carious lesions approaching the pulp, asymptomatic or with symptoms of dentinal pain like sensitivity to cold or sweet, as described by the parents or the child. Radiographically, carious lesions depth to be at least two thirds of dentin thickness, with no furcation or periapical radiolucency. Exclusion criteria consisted of primary molars with history of spontaneous pain, advanced pulp degeneration or necrosis (e.g., swelling, suppuration, purulence) and/or radiographic signs of furcation or periapical pathology, internal or external root resorptions. Teeth with previous restorations were as well, excluded from the study.

For the pulpectomy group, inclusion criteria consisted of primary molars with extensive clinical and radiographic caries, symptoms of irreversible pulpitis: spontaneous pain or pain not relieved by analgesics, no history of abscess or the use of antibiotics for treating the infected tooth. Radiographically, roots show minimal or no resorption. [30]

Occlusal and proximal carious lesions were included in the study. For proximal lesions with pain suspected as from food impaction, final diagnosis was obtained during treatment based on bleeding parameters. Teeth with hyperemic pulps were treated with pulpectomy even in the absence of symptoms. Teeth diagnosed with pulp necrosis or partial necrosis were excluded from the study.

Ethical considerations

Study protocol was approved by the Institutional Human Subjects Ethics Committee of Hadassah Medical Organization, Jerusalem, Israel. All procedures performed were in accordance with the ethical standards of the Institution and National Research Committees with no compensation offered. A detailed information fact sheet in simple, non-technical language, was provided in advance. Parents/guardians of all patients included in the study were requested to sign an informed consent form. The study protocol can be accessed at clinicaltrials.gov (NCT01486537).

Samples collection

After local anesthesia with 2% Xylocaine Dental with epinephrine 1:100,000 (lidocaine HCl and epinephrine Injection, Dentsply Pharmaceutical, York, PA, USA) and rubber dam isolation, cavity preparation was performed using a high-speed 330 Standard Operatory Carbide Bur (SS White® Burs, Inc. Lakewood, NJ, USA) under air–water coolant. Dental caries was removed using low-speed, round steel burs (Emil Lange, Engelskirchen, Germany) prior to accessing the pulp chamber. After carious pulp exposure, pulp chamber was unroofed with a #330 bur, and coronal pulp tissue excised using a sterile, low speed round bur. At this phase, blood was collected by the operator using a sterile 100 microliter syringe pretreated with heparin solution (Heparin Injection BP 5,000 UNITS/ML, Rotexmedica, Trittau, Germany). The operative decision of performing a pulpotomy or pulpectomy was based on bleeding color and whether hemostasis was obtained within 5 minutes with a cotton pellet placed inside the pulp chamber with

light pressure. When pre-operative history of spontaneous pain existed, difficulty in obtaining hemostasis and/or presence of dark purple blood, pulpectomy was performed. Clinical and radiographic findings and the behavior guidance technique utilized during treatment were documented.

Within ten minutes from collecting the pulpal blood sample, a neonate Astrup (high accuracy technique for determining the acid-base values of blood based on the equilibration principle),[29,30] was performed using a glass capillary at the pediatric intensive care unit of the Medical Center (storage not needed). The Astrup method for determination of arterial pH, pCO_2, and "base excess" provides a simple and accurate means for quantitation of acid-base disorders.[17] This method for determination of blood gas module pH, pCO_2, and pO_2 was performed using cobas b 221 POC system (Roche Diagnostics Ltd. CH-6343 Rotkreuz Switzerland) and time to result was less than 2 minutes with whole-blood sampling.

Statistical analysis

Student's t-test and the non-parametric Mann Whitney test or independent 2-group Mann-Whitney U Test were used for data analysis. The correlation between each two quantitative parameters (e.g. age and pH, pCO_2, pO_2) was estimated by calculating Pearson correlation coefficient. The comparison of pO_2 and pCO_2 values in pulpotomized teeth to pO_2 and pCO_2 values in pulpectomized teeth was performed using One-sample t-test. The software used for statistical analysis was IBM® SPSS® Statistics version 20 for Windows and level of significance set at $p< 0.05$. Results presented as the mean ± standard error.

RESULTS

Eighty-four children participated in the study (37 girls and 47 boys). Average age was 5.2 (SD = 1.6) year old and median age 4.7 without any differences between pulpotomy or pulpectomy groups. Seventy-one children (84%) were treated with the aid of inhalation analgesia (nitrous oxide), conscious sedation (oral midazolam, hydroxyzine or a combination of the two) and general anesthesia. Twenty-three teeth were first primary molars and 61 teeth were second primary molars Fourteen (17%) teeth were maxillary and 70 (83%) were mandibular. Pulpotomy was performed on 58 teeth (69%) and pulpectomy on 23 (27 %). A chi-square test of independence showed that there was a significant association between tooth type and the *treatment performed (p=.04, df =1) Table1.*

Table 1: treatment by tooth type.

	Pulpotomy	Pulpectomy	Total
1st primary molar	20	3	23
2nd primary molar	38	23	61
Total	58	26	84

*chi-square test of independence showed that there was a significant association between tooth type and the treatment performed (p=.04, df=1)

Acidity level

Pulpotomy samples had a tendency toward a more basic pH when compared to the pulpectomy ones. The average pH for teeth treated with pulpotomy was 7.535, while 7.508 for pulpectomy with no significant difference (P= 0.137).

Gas tension

The average pO_2 for teeth treated with pulpotomy was 175.6 mmHg, while the average pO_2 for pulpectomy was 178 mmHg but with no significant difference (P= 0.245), The average pCO_2 for teeth treated with pulpotomy was 9.4 mmHg, while for the pulpectomy group the average was 13.4 mmHg. This was statistically significant (P= 0.002).

Behavior guidance

Twelve children were treated using local anesthesia alone, 62 under nitrous oxide and conscious sedation, and 10 under general anesthesia. None of the behavior guidance techniques influenced the pH ($p = 0.630$) pO_2 ($p = 0.481$) and pCO_2 ($p = 0.150$).

Type of tooth

Twenty-three were first primary molars and 61 second primary molars. Type of treated tooth had no significant effect on blood samples pH, pO_2 or pCO_2 (Table 2).

Table 2: pH/pO2/pCO2 in accordance to procedure and tooth type

		pH	P value	pO2 (mmHg)	P value	pCO2 (mmHg)	P value
1st primary molar	**Pulpotomy**	7.573 SD = 0.069	0.25	179.32 SD = 15.26	0.56	9.157 SD = 4.222	0.72
	Pulpectomy	7.492 SD = 0.106		182.633 SD = 12.592		10.766 SD = 7.44	
2nd primary molar	**Pulpotomy**	7.516 SD = 14.852	0.93	173.738 SD = 14.852	0.1	9.786 SD = 3.939	0.03**
	Pulpectomy	7.509 SD = 0.063		179.58 SD = 16.055		13.704 SD = 6.79	

**According to independent 2-group Mann-Whitney U Test

DISCUSSION

Clinicians face a major diagnostic challenge in deciding whether the inflammatory state of the pulp is reversible,[3] a diagnosis that in turn will determine tooth prognosis.[3] In an effort to find objective clear-cut parameters that differ significantly between reversibly and irreversibly damaged pulps, we measured acidity level and blood partial pressure of O_2 and CO_2 of the pulp in primary molars that underwent either pulpotomy or pulpectomy treatment. We can conclude that both acidity level and blood partial pressure of O_2 are not adequate indicators of pulp inflammatory state. However, significantly higher pCO2 values were found in teeth scheduled for pulpectomy. High carbon dioxide pressure is a result of inflammatory environment. CO_2 can combine reversibly with H_2O to yield a strongly acidic H+ ion and a weak basic bicarbonate ion (HCO_3). CO_2/HCO_3 serves to buffer the system and induce pH recovery after induced-intracellular alkalosis.40 We assume that, in severely inflamed pulps, the extracellular high levels of CO2 serve to cope with the inflammatory state and may contribute to the pulp's pH tendency towards alkalosis.

The pH of a normal human dental pulp tissue is 7.4 ± 0.2.[33] We hypothesized that the pH of an inflamed pulp will be more acidic than that of a healthy one, since low extracellular pH (i.e., tissue acidosis) is frequently seen in inflamed tissue.[12] Our results demonstrated that in both groups, pulpotomy and pulpectomy, pH was within normal range but in inflamed tissue (teeth that were diagnosed with irreversible pulpitis and underwent pulpectomy) it was slightly more acidic than in the pulpotomy group. Although this finding was statistically non-significant, it is interesting to note that the pulp's tendency towards alkalosis is a part of its regeneration process, as higher intracellular pH serves as a permissive or obligatory signal for cell proliferation.[27] Okabe *et al* have shown that human dental pulp cell mineralization is enhanced in alkaline pH conditions: both alkaline phosphatase (ALP) activity and quantity of bone morphogenetic protein 2 (BMP-2) were increased at pH 7.8 compared with cells cultured in pH 7.2 conditioned medium.[34] BMP-2 accelerates the differentiation of human dental pulp cells into odontoblasts and increases the activity ALP,[25] a known indicator of osteoblast differentiation and osteogenesis.[32]

The highly constricted anatomy of the dental pulp leads to high capillary pressures.[33] Normal pulpal capillary pressure is relatively high (35 ± 0.8 mmHg),[35]and pulpal interstitial fluid pressure (IFP), in contrast to most other tissues, is well above atmospheric pressure (6–60 mmHg).[2] During inflammation, the IFP of dental pulp increases approximately threefold.[2] The values of gases partial pressures in our study are higher than values found in blood capillaries, where pCO_2 is 35.00˜45.00mmHg and pO_2 is 75.00˜100.00 mmHg.[32,36,37] The literature lacks agreement between results on tissues pO_2 distribution.[37] Differences are introduced by the method for exposing the tissue, the variability between tissue, differences in measurement methods and the intrinsic potential variability due to the location of the measurement site along the microvasculature.[37] Therefore, we compared the values measured between the two groups of teeth and more studies are needed to provide further data on pulp gases partial pressures.

The slightly higher pO_2 measured in samples from teeth that underwent pulpectomy in comparison to those treated with pulpotomy may be explained by the fact that tissues in a state of acute inflammation present vasodilation and increased vascular permeability that allows leukocytes entrance into the pulp tissue.[10,18] The average pO2 for teeth treated with pulpotomy was 175.6 mmHg, while the average pO2 for pulpectomy was 178 mmHg with no significant difference. This corroborates with the literature.[36]

No difference was found between maxillary and mandibular teeth. Difference was found between first to second molars regarding pH, but not regarding the other parameters that were measured (pO_2 and pCO_2).

Our results showed that, in an inflamed pulp tissue, the partial CO_2 pressure is higher than in healthy pulp tissue, pH is slightly more alkalosis and partial O_2 pressure is slightly higher. These small differences between reversibly and irreversibly damaged pulp tissues demonstrate the proximity of the two conditions, rendering precise diagnosis of pulp condition very difficult. The present study is a step towards finding further measures that will shed light on the fine transition from reversible inflammation state to irreversibly damaged pulp tissue.

Study Limitations: Diagnosis and treatment decisions were based on history of pain and symptoms and were made upon evaluation of the pulp status after exposure. Even though criteria for performing pulpotomy or pulpectomy were clear, diagnostic errors might have occurred. The rationale for this study was to seek a more specific method for pulp diagnosis in situations where pre-operative pain and symptoms history are unclear, and bleeding is present after pulp exposure. Further investigations with larger numbers of teeth are needed in order to support the findings of this study.

CONCLUSIONS

The following conclusions were drawn from the study:

1. Significantly higher pCO_2 levels were found in pulps needing pulpectomy;
2. Although not statistically significant, more acidic pH could be found in pulps needing pulpectomy.

DECLARATIONS

Ethics approval and consent to participate

Study protocol was approved by the Institutional Human Subjects Ethics Committee of Medical Organization IRB (0401-11-HMO).

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent was obtained from all parents or caregiver of the participants included in the study.

Competing interests

The authors declare that they have no competing interests

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