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Effects of Age, Sex, and Blood Pressure on the Blood Flow Velocity in Dental Pulp Measured by Doppler Ultrasound Technique

Short Title

Systemic Factors and Pulpal Blood Flow Velocity

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The authors declare that they have no conflict of interest.

ABSTRACT

Objective: To examine the effects of age, sex, and BP on the PBFV in human maxillary anterior teeth.

Methods: A total of 332 maxillary anterior teeth from 95 participants were included. The age and sex of each subject were recorded. The systolic and diastolic BP was measured by a digital sphygmomanometer. The PBFV of each tooth was measured by using a Doppler ultrasound system with a 20 MHz-transducer.

Results: Among the variables, only systolic BP demonstrated a significant association with the PBFV ($p < 0.001$). There were significant decreases in the PBFV with the groups of lower BP ($p < 0.05$). Age, sex, and tooth type were not significantly associated with the PBFV.

Conclusions: Within the limitations of this study, it is considered that the PBFV increases with an increase in systolic BP. Age, sex, and tooth type had no significant effect on the PBFV of maxillary anterior teeth.

Key words: Dental pulp, Pulpal blood flow velocity, Doppler ultrasound, Blood pressure, Systemic factor

LIST OF ABBREVIATIONS

BP, blood pressure

CW, continuous-wave

LDF, laser Doppler flowmetry;

PBF, pulpal blood flow

PBFV, pulpal blood flow velocity;

SD, standard deviation

Vam, mean linear velocity from the average velocity curve of a Doppler spectrum

INTRODUCTION

The main functions of microcirculation are the delivery of oxygen and nutrients to tissue and the removal of carbon dioxide and waste products. Vitality of the tissue is highly dependent on adequate microcirculation, and alterations in microcirculatory function may cause pathological processes and result in tissue dysfunction [9]. Therefore, in order to understand the progression of diseases, we should be aware of not only the anatomical structures but also the physiological regulatory mechanisms of microcirculation in the tissue of interest. In the oral cavity, periodontal tissues are relatively well studied with regard to the microcirculation. It is thought that inflammation in the periodontal tissues results in the breakdown of microcirculatory function, and that blood flow may serve as a predictor of periodontal disease [13]. However, there is limited information about the dental pulp, particularly in human teeth.

The microcirculation of dental pulp has its own unique structural characteristics. The dental pulp is a loose connective tissue surrounded by avascular and highly calcified hard tissues—enamel, dentin, and cementum. It receives blood supply from arterioles that enter the tooth through small apical foramina located at the end of the root. The compliance of the dental pulp is quite low, and the total volume of blood within the pulp space cannot be greatly increased. The rigid calcified shell provides protection for the pulp tissue from the outer oral environment. However, it makes the study of the microcirculation difficult, because the pulp is inaccessible unless a hole is made through the hard tissues [28]. Furthermore, the accurate clinical assessment of pulp status is complicated. Although measurement of PBF would be an ideal tool for determining pulp vitality, only sensitivity tests such as thermal or electric pulp testing, which examine the nerve response to a stimulus, are available in clinics. Theses sensitivitiy tests often produce false results, particularly in healthy immature teeth [12], traumatized teeth [1], or teeth undergoing orthodontic treatment [35].

There have been several studies of the microcirculation of the dental pulp. The vascular architecture of the dental pulp was confirmed in early histological studies. The arterioles run axially in the center of the pulp, whereas venules are located in the periphery [25]. Shunt vessels such as arteriovenous anastomoses, venovenous anastomoses, and U-turn loops, which provide a direct communication between the arterioles and venules, are also observed in the pulp [38]. The diameters of blood vessels are less than 100 μm, and vary according to the tooth and location within the same tooth [7,34]. Animal studies have used invasive methods such as the radioisotope clearance test [16] and hydrogen gas desaturation test [39] for a direct assessment of PBF. It was confirmed that the pulp is one of the most highly vascularized tissues of the body [42], and the PBF is estimated to be relatively high compared to other tissues [23]. Kim et al. [24] used intravital microscopy to study the profiles of PBF and reported that the PBFV was five times higher in the arterioles than in similar-sized venules. The regulation of PBF was found to be controlled by systemic blood pressure (BP) [36,40], as well as by neuronal, local, and humoral mechanisms [2,29,32].

In contrast to those in animals, studies regarding PBF in human teeth have been limited. LDF has been used for measurement of PBF through the hard tissues [10,22]. However, the signals are easily affected by the backscattered light from periodontal tissues, and any obstruction of the light pathway can render the technique useless [15]. The results are obtained as relative proportions, which cannot be calibrated in absolute units [43]. Recently, researchers have utilized Doppler ultrasound for measurement of PBF. This offers obvious advantages for the estimation of blood flow, as it directly measures the frequency changes in ultrasound reflected from red blood cells [33]. By using a Doppler ultrasound device, the PBF of vital teeth can be distinguished from root canal-filled teeth [44]. Changes in the PBFV can be identified after infiltration with an anesthetic agent containing epinephrine [45]. The mean PBFV of normal maxillary anterior teeth was successfully measured and found to be approximately 0.56 cm/s regardless of tooth type [8]. These results imply that Doppler ultrasound can be an effective clinical diagnostic tool for the dental pulp. Considering that they only included intact teeth of young and healthy subjects, the change in the PBF according to the alteration of systemic variables such as age and blood pressure, as well as local factors such as dental caries and pulpitis, should be also investigated.

It is known that there are age-related anatomical and functional changes in the microcirculation [3]: increased stiffness, decreased density, impaired organization, and decreased reactivity. The dental pulp also demonstrates age-related changes similar to other parts of the body [31]. The lifelong deposition of secondary dentin and cementum tends to narrow the apical foramen and pulp space, which compromises the supply of blood vessels. There is a decrease in the number of blood vessels, as well as arteriosclerotic changes in the vessels that supply the pulp of aging teeth [4,5]. The arterial intima thickens and the adventitia calcifies [21]. A clinical study reported a significant decrease in the resting PBF with increasing age of the participants [17]. The changes in PBF in response to a temperature shift were also smaller in older subjects.

Some systemic diseases, such as hypertension, affect the microcirculation. In hypertension, the mechanisms regulating vasomotor tone become abnormal, the structure of individual precapillary resistance vessels is altered, and the density of arterioles or capillaries can be reduced [27]. The association between hypertension and periodontitis has been suggested and actively researched [26,41]. However, there is no available literature on the relationship between blood pressure and pulpal status.

To summarize, the knowledge about PBF in human teeth is limited due to structural issues, and more clinical information is needed. The purpose of this study was to examine the effects of age, sex, and systemic BP on the PBFV in human maxillary anterior teeth. A Doppler ultrasound device was used for the quantitative measurement of PBFV.

MATERIALS AND METHODS

Participants

A total of 332 maxillary anterior teeth from 94 subjects (age range: 11 to 70 years) were included in the present study. The study was performed in the Department of Conservative Dentistry, Yonsei University Dental Hospital, Seoul, South Korea, between March 2012 and December 2014. Participants were selected from among the patients who visited the clinic for dental treatments or regular follow-ups. The age and sex was recorded, and a clinical dental examination was performed for each subject, including tooth vitality test, mobility and percussion test, and measurement of periodontal pocket depth. Subjects with teeth which met the inclusion and exclusion criteria were initially assessed for eligibility in the study, and written informed consent was obtained from each participant before enrollment in this study.

The inclusion criteria were as follows:

1) Maxillary anterior teeth, including central and lateral incisors and canines;
2) Teeth with no dental caries, abfractions, or previous restorations;
3) Teeth with no history of orthodontic treatment or dental trauma;
4) Teeth with normal responses to thermal stimuli; and
5) Teeth with no sensitive response to percussion and mobility of less than 1 mm.

The exclusion criteria were as follows:

1) Teeth diagnosed with pulpitis or pulp necrosis;
2) Teeth with previous or ongoing root canal treatment;
3) Teeth with chronic advanced periodontitis, which shows more than 6 mm of pocket depth; and
4) Teeth with any clinical signs and/or symptoms.

This study was approved by the Yonsei University Committee for Research on Human Subjects (2-2010-0002). All procedures were in accordance with the ethical standards of the institutional committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Measurements

All procedures were performed by experienced faculty and residents in the clinic. Each subject was provided 10 minutes of rest prior to the measurement, seated in a semisupine position. The systolic and diastolic BP was measured and recorded using a digital sphygmomanometer (Angelus; KTMED, Seoul, South Korea). Following this, the PBF of each individual tooth was examined by using a Doppler ultrasound device (MM-D-K; Minimax Ltd., St. Petersburg, Russia) (Figure 1). A 20-MHz CW transducer with a 1.5-mm sensor diameter was connected to a computer. After drying the tooth surface, a small amount of ultrasonographic coupling gel (Pro-gel II; Dayo Medical, Seoul, South Korea) was applied prior to the measurement. The transducer was positioned on the labial surface of the tooth, at an angle of approximately 60° with respect to the longitudinal axis of the tooth. The examiner searched for the signal by listening for pulsating sounds. The transducer was placed at the site with the clearest sound, and was held for about 10 seconds so that the signals could be recorded steadily and with consistency. Once the measurement was obtained, the Doppler spectra were recorded and the flow indices were calculated using the Minimax Doppler 1.71 (Minimax, St. Petersburg, Russia) software program (Figure 2).

Statistical analysis

The unit of analysis was the tooth, with the subject being the cluster. The PBFV was a dependent variable, and the Vam from each Doppler spectrum was used for the analysis as described in a previous study [8]. Age, sex, systolic BP, and tooth type were examined for significance in a generalized linear model analysis. This model took account of the clustering effect of multiple teeth within subjects. All statistical analyses were performed with a 95% confidence level, using SPSS 23 (IBM Corp., Somers, NY, USA) software.

RESULTS

The baseline characteristics of the subjects and teeth are summarized in Table 1 and 2. Of the subjects, 45 (47.4%) were males versus 50 (52.6%) females. The mean age was 29.6 ± 11.7 (SD) years, and the mean systolic BP was 117.0 ± 15.3 (SD) mmHg. Thirteen subjects (13.7%) showed hypotensive BP (≤ 100 mmHg), 44 (46.3%) showed normotensive BP (101-120 mmHg), 42 (32.6%) showed prehypertensive BP (121-140 mmHg), and 7 (7.4%) showed hypertensive BP (> 140 mmHg). Over all teeth, central incisors, lateral incisors, and canines were 92 (27.7%), 138 (41.6%), and 102 (30.7%), respectively.

Table 3 presents the significant variables related to the PBFV based on the generalized linear model ($r = 0.252$, $p < 0.001$). Among the variables, only systolic BP demonstrated a significant association with the PBFV ($p < 0.001$). Using the Vam with hypertensive BP (> 140 mmHg) as a reference, the PBFV with prehypertensive BP decreased ($p = 0.042$). The PBFV continued to decrease for normotensive BP ($p = 0.002$), and still more for hypotensive BP ($p = 0.001$). Age, sex, and tooth type were not significantly associated with the PBFV.

DISCUSSION

In this study, the data were analyzed for associations between age, sex, systolic BP, and tooth type and PBFV, using a multivariate linear model. The analysis was carried out at tooth level, accounting for clustering in the data. As multiple teeth were included from each participant, the clustering effect was considered with the subject being the cluster and the individuals the teeth nested within subjects. The main finding of this study was that the PBFV was associated with systolic BP. There were significant decreases in the PBFV with the groups of lower BP ($p < 0.05$) (Table 3).

Although several experimental studies have reported microvascular structural alterations in hypertensive models of other organs [18], the effect of BP on the pulpal microcirculation remains uncertain, as there is no comparable literature about the relationship between the BP and PBF in humans. In other medical fields, some studies have investigated the effect of BP on peripheral blood flow. The results differed depending on the target tissue. Grunwald et al. [14] showed that glaucoma patients with systemic hypertension had higher optic nerve blood flow than patients without hypertension. Esgin et al. [11] measured the pulsatile ocular blood flow of diabetic patients, and the results suggested that subjects with systemic hypertension had an increased blood flow rate compared to controls without hypertension. In contrast, Kanoore Edul et al. [20] said that there was no correlation between BP and blood flow in the sublingual microcirculation. In another study, the blood flow velocity decreased with higher BP in the small vessels of the skin [19]. In the present study, we tentatively classified the participants into four groups according to systolic BP at intervals of 20 mmHg. The comparison of the PBFV between subjects diagnosed with hypertension and those without hypertension should be considered in further investigations.

A previous study reported a negative relationship between the age and PBF in human teeth. Ikawa et al. [17] examined age-related changes in human PBF by using the LDF, and reported a significant decrease of PBF with increasing age. Histological findings also indicate decreased PBF with aging [4,5,31]. However, in the present study, the age was not found to be associated with the PBFV ($p = 0.694$) (Table 3). These conflicting results might be due to that the previous study did not consider the correlation between age and BP. Generally, elders have high BP. In this study, there was a significant correlation between the age and BP of the subjects ($r = 0.507$, $p < 0.001$). The multivariate model identified only systolic BP as a determinant (Table 3). It refers that the PBFV was influenced by BP rather than the age of subject.

Tooth type had no significant effect on the PBFV, and this was in agreement with a previous study that analyzed the mean PBFV of 359 maxillary anterior teeth [8]. The mean PBFV was 0.508 cm/s in this study, which was roughly comparable to the results of the previous study (0.56 cm/s). However, the wide distribution of the measured PBFV might be a limitation of both studies. There could be several factors that influence the measurement of PBFV. In our preliminary study, the measured PBFV revealed large deviations among patients and between examiners. The measurements were performed by several different examiners in this study. It might have been more consistent if the data had been obtained by a single examiner. It is known that the angle and position of the transducer can affect the results [37]. Even a little difference in the angle can make a significant change in the estimated velocity, especially at large angles. In the present study, the examiners were trained to make the angle at approximately 60º with respect to the longitudinal axis of the tooth, which is considered as the maximal angle in clinical practice. Though we tried to minimize the effects of Doppler angle, it is likely that it could cause some errors. Lastly, the layers of enamel and dentin show natural variations among individuals, tooth types, and sites on specific teeth. Therefore, the scatter, reflection, and attenuation of ultrasound will be different for each tooth, thus affecting the velocity estimation of the Doppler ultrasound device.

It was suggested that high-frequency Doppler ultrasound has the potential to play an important role in examining the PBF of teeth in both clinical and research settings [6]. The spatial resolution of an ultrasound device increases proportionally with the center frequency of the system. Therefore, by using high-frequency transducers, dental structures should be resolved in higher detail. However, Doppler ultrasound is not generally available for clinical use in dentistry. The MM-D-K, which was used in this study, is the only Doppler ultrasound device that is currently usable for dental use. It uses a high-frequency CW transducer to assess the dynamics of the blood flow in microvessels. In this study, we used the 20 MHz-CW transducer, which is recommended for detecting vessels at 0.1-8 mm depth, according to the manufacturer's instructions (Figure 1). It has a sensor with a small diameter (1.5 mm) so that it is possible to position it in a tight area such as the tooth surface. Continued improvement in the clinical performance of Doppler ultrasound devices for dental use is needed, and further development and research of various types of transducers is necessary. Additionally, a technique of estimating the volume of moving blood in the pulp should be applied for more relevant information regarding the pulp physiology.

The population in this study was limited to patients in our department and controlled by inclusion and exclusion criteria. These factors could affect the BP distribution of the participants. The mean systolic and diastolic BPs of the Korean population in 2013 were 120.1 and 78.5 mmHg in males and 116.6 and 73.4 mmHg in females, respectively [30]. The prevalence rate of hypertension was 30.4%. In the present study, the mean systolic and diastolic BPs were 121.5 and 72.6 mmHg in males and 113.0 and 69.3 mmHg in females. These values are comparable to the general Korean population. The proportion of subjects with hypertensive BP (more than 140 mmHg, 7.4%) was lower than the prevalence rate of hypertension.

Within the limitations of this study, age, sex and tooth type had no significant effect on the PBFV of maxillary anterior teeth. Only systolic BP was correlated with PBFV, and PBFV increased with an increase in BP. This is the first report of the relationship between systemic factors and PBFV in humans, using Doppler ultrasound technique. This noninvasive and quantitative measurement technique may enable the study of pulpal microcirculation under physiological conditions, as well as the changes that occur in pathologic conditions. Longitudinal observations with repeated measurements of the PBFV may provide more clinically relevant information about pulp physiology. Further investigations are required to clarify the effects of other systemic factors or diseases, such as smoking, diabetes, cardiovascular diseases, and genetic disorders, as well as local factors such as dental caries, attrition, restoration, periodontal disease, and pulpitis, on the PBFV.

PERSPECTIVES

PBFV was correlated with systolic BP, and PBFV increased with an increase in BP. This is the first report of the relationship between systemic factors and PBFV, using Doppler ultrasound technique. This study can provide a basis for future research associating PBFV with various local and systemic factors, as well as for research on the hemodynamics and physiology of dental pulp.

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The authors deny any conflicts of interest related to this study.

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TABLES

Table 1. Baseline characteristics of the subject-related variables (N = 95)

	N	**% / Mean ± SD**
Sex		
Male	45	47.4%
Female	50	52.6%
Age (years)		29.6 ± 11.7
-20	20	21.0%
21-40	51	53.7%
41-	24	25.3%
Systolic BP (mmHg)		117.0 ± 15.3
Hypotensive (≤ 100)	13	13.7%
Normotensive (101-120)	44	46.3%
Prehypertensive (121-140)	31	32.6%
Hypertensive (> 140)	7	7.4%

SD, standard deviation; BP, blood pressure

Table 2. Baseline characteristics of the tooth-related variables (N = 332)

	N	**%**
Tooth type		
Central incisor	92	27.7%
Lateral incisor	138	41.6%
Canine	102	30.7%

Table 3. Generalized linear model analysis for the PBFV (N = 332)

	Parameter Estimate	SE	95% CI		*p*
			Lower	Upper	
Sex					
Male	0.102	0.069	-0.035	0.238	0.141
Female†					
Age (years)	0.001	0.003	-0.005	0.007	0.694
Systolic BP (mmHg)					
Hypotensive (≤ 100)	-0.611	0.173	-0.955	-0.267	0.001**
Normotensive (101-120)	-0.555	0.173	-0.898	-0.211	0.002**
Prehypertensive (121-140)	-0.363	0.176	-0.713	-0.013	0.042*
Hypertensive (> 140)†					
Tooth type					
Central incisor	-0.067	0.036	-0.139	0.004	0.066
Lateral incisor	-0.044	0.034	-0.111	0.023	0.192
Canine†					

SE, standard error; CI, confidence interval; BP, blood pressure

Reference group

* $p < 0.05$; ** $p < 0.01$

FIGURE LEGENDS

Figure 1. 20 MHz Transducer of the Doppler ultrasound device (Minimax Ltd., St. Petersburg, Russia)

(A) Tip of the transducer

(B) Transducer held in a hand

(C) Positioning of the transducer on a central incisor

Figure 2. Doppler spectra and flow indices from the Minimax Doppler 1.71 software program (Minimax Ltd., St. Petersburg, Russia)

Vs, systolic peak velocity from the maximum velocity curve; Vas, systolic peak velocity from the average velocity curve; Vm, mean velocity from the maximum velocity curve; Vam, mean velocity from the average velocity curve; Vd, end diastolic velocity from the maximum velocity curve; Vad, end diastolic velocity from the average velocity curve; PI, pulsatility index; RI, resistivity index; and STI, systolic to diastolic ratio

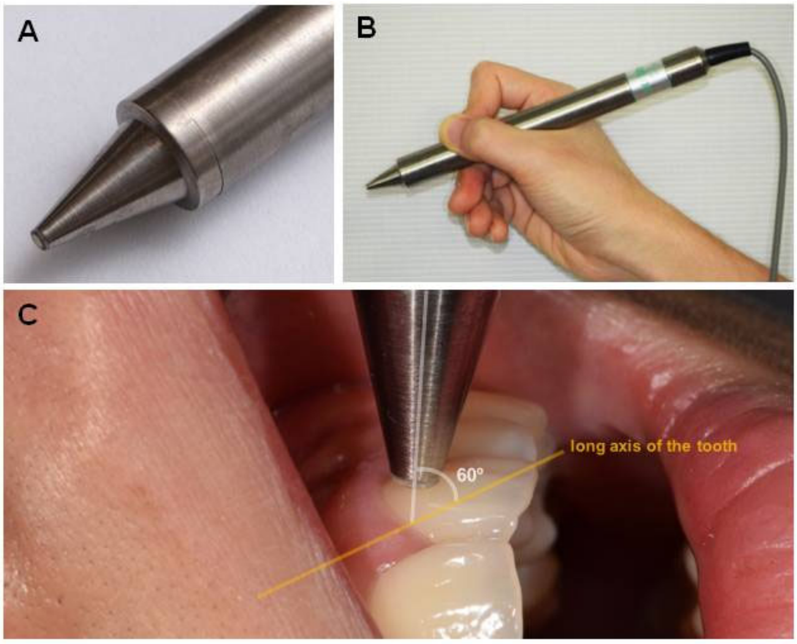

Figure 1

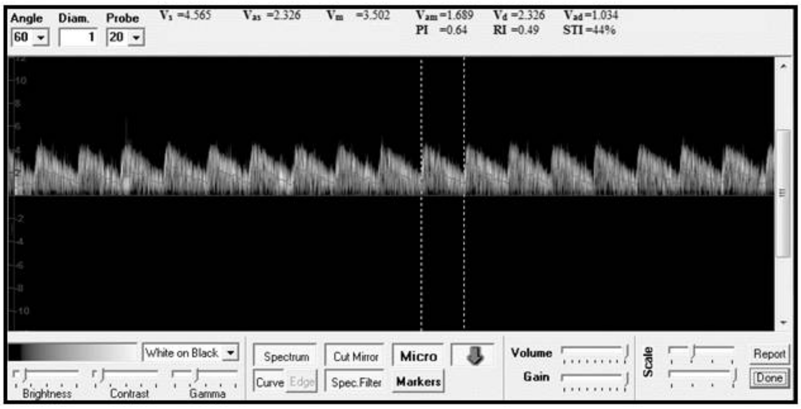
Angle
Diam.
Probe
60
1
20
Vs =4.565
Vas =2.326
Vm =3.502
Vam =1.689
PI =0.64
Vd =2.326
RI =0.49
Vad =1.034
STI =44%
White on Black
Brightness
Contrast
Gamma
Spectrum
Cut Mirror
Micro
Curve
Edge
Spec.Filter
Markers
Volume
Gain
Scale
Report
Done

Figure 2