Three-dimensional volumetric magnetic resonance imaging (MRI) analysis of the soft palate and nasopharynx in brachycephalic and non-brachycephalic dog breeds

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ABSTRACT. The purpose of this study was to use magnetic resonance imaging (MRI) to compare the volumes and three-dimensional configurations of the soft palate and nasopharynx in non-brachycephalic and brachycephalic dogs with different body weights, and infer which factors influence nasopharyngeal volume. This was a retrospective observational study. The brain MRI medical records of all dogs referred to the Veterinary Medical Center, Chungbuk National University, between 2013 and 2016, for evaluation of intracranial disease were reviewed. There was a significant difference in the two-dimensional parameters including soft palate length/skull length ratio (P<0.01) and maximum soft palate thickness (P<0.01), and three-dimensional parameters which included soft palate volume (P<0.01), nasopharyngeal volume (P<0.01), soft palate/total upper airway volume ratio (P<0.01), and nasopharyngeal volume/total upper airway volume ratio (P<0.01), between brachycephalic and non-brachycephalic dog breeds. Nasopharyngeal volume correlated positively with the maximum soft palate thickness and body weight in all breeds. The three-dimensional morphologic grades of soft palate were significantly different between the two groups. In brachycephalic breeds, Grade 3 was observed in 33% of cases but was absent in non-brachycephalic breeds, where Grade 1 was present in 85% of the cases. We can conclude that three-dimensional morphology and upper airway volume are significantly different between brachycephalic and non-brachycephalic breeds, and body weight and maximum soft palate thickness are the key factors associated with a decreased nasopharyngeal volume.

KEY WORDS: brachycephalic airway syndrome, brachycephalic canine breed, nasopharyngeal anatomy

Brachycephalic dog breeds are popular breeds that present with specific craniofacial anatomical abnormalities. In brachycephalic breeds, the upper jaw is hereditarily shorter in proportion to body size while the lower jaw is of normal length. All the oral soft tissue is still present in the shortened maxilla, resulting in an over-crowed upper airway area. This malformed craniofacial morphology leads to anatomical obstruction of the upper airways and increased airway resistance [12, 15].

Brachycephalic airway syndrome is a condition that most brachycephalic breeds experience. This syndrome usually includes anatomic characteristics such as stenotic nares, elongated soft palate, hypoplastic trachea, and nasopharyngeal turbinates [13]. These primary anatomic components create a negative pressure within the airway because of increased respiratory effort, leading to additional, secondary problems, such as everted laryngeal sacculae, laryngeal collapse, and everted tonsils, which create further airway turbulence and edema of the pharyngeal tissue through increased respiratory efforts [8].

Many efforts have been made in veterinary science to standardize the evaluation of brachycephalic airway syndrome. Computed tomography (CT) is one of the most popular modalities currently used for assessment of upper airway anatomy in dogs nowadays. One report investigated the unique craniofacial structures of brachycephalic breeds using CT [14]. Another report recently showed that the thickness of the soft palate is the most important component of severe brachycephalic airway syndrome [10].

Pharyngeal collapse is a complex disease that is not likely a primary disorder and more likely to be secondary to long-term...
negative pressure gradients [19]. Brachycephalic dogs experience long-term negative pressure gradients associated with increased inspiratory upper airway resistance resulting in a compressed nasal passage and altered pharyngeal anatomy. This condition makes brachycephalic dogs more susceptible to pharyngeal collapse.

Magnetic resonance imaging (MRI) provides superior resolution of soft tissues composing the upper airway; it is accurate, reproducible, and free of ionizing radiation. Contrary to MRI, CT has lower histological resolution and this results in difficulty to distinguish the soft palate from surrounding tissue particularly for transaxial images. This technique has been widely validated in human beings with obstructive sleep apnea syndrome (OSAS) to obtain volumetric measurements of the upper airway and the tissues comprising the airway [2]. One study showed that the volume of the soft palate was larger and total upper airway volume was significantly smaller in children with OSAS [1]. However, MRI has not been used in the past to systematically evaluate the upper airway in brachycephalic dogs.

The purpose of this study was to use MRI to compare the volume and the three-dimensional configuration of the soft palate and nasopharynx in non-brachycephalic and brachycephalic dogs with different body weights and infer which factors influence nasopharyngeal volume.

We hypothesized that the upper airway and soft palate volume as assessed by MRI in brachycephalic dogs would be different from that in non-brachycephalic dogs. We also hypothesized that the prevalence of the grades of three-dimensional classification would be different between the two groups. Moreover, body weight, soft palate thickness and volume would have an influence on nasopharyngeal volume.

MATERIALS AND METHODS

**Inclusion criteria**

All dogs that underwent brain MRI at the Veterinary Medical Center, Chungbuk national university between December 2013 and December 2016 were reviewed retrospectively. Brachycephalic dog breeds included the Shih Tzu, Pekingese, Pomeranian, Chihuahua, Cavalier King Charles Spaniel, and Pug while non-brachycephalic dog breeds included the Poodle, Bichon Frise, Spitz, Miniature Schnauzer, and Dachshund. Dogs which are not typically brachycephalic but meet the criteria for cephalic index of brachycephalic dogs were considered as brachycephalic dog [7]. For example, Pomeranians were included given their cephalic index although there are not representative of brachycephalic dogs. Pomeranian was known for predisposing to brachycephalic airway syndrome [16]. Another article associated with brachycephalic syndrome included two Pomeranians [18]. Dogs with body weights over 10 kg or less than 3 kg were excluded to avoid excessive variation in anatomical morphology.

**Imaging technique**

Imaging was performed using a low-field MRI system (0.3T, Airis II; Hitachi, Tokyo, Japan) with a 0.3T joint coil. T2-weighted sagittal and transverse images were evaluated and cases with missing sequences were excluded. Sagittal T2-weighted images were obtained with a repetition time of 2,500 to 4,000 msec and an echo time of 90 msec. Transverse T2-weighted images were obtained with a repetition time of 2,500 to 5,000 msec and an echo time of 120 msec. Slice thickness was 3 mm with an interslice gap of 0.5 mm for transverse images.

**Image evaluation**

Mid-sagittal images were employed to obtain two-dimensional parameters. Skull length (SL) was defined as the distance from the rostral limit of the nostril to external occipital protuberance. The length of the soft palate was measured from the end of the hard palate to the caudal end of the soft palate. The thickness of the soft palate was measured perpendicular to this line and the maximum value was recorded (Fig. 1). The cross-sectional area (CSA) of the nasopharynx was defined as the air passage extending from the caudal end of the choanae to the caudal free border of the soft palate. Cross-sectional area of the soft palate was also obtained in the mid-sagittal view (Fig. 1). To obtain three-dimensional parameters, both mid-sagittal and transverse views were employed. The volumes of the soft palate and nasopharynx were obtained by adding up all the CSA calculated for each transverse image (Fig. 2). To adjust for differences in size among the dogs, all two and three-dimensional parameters were adjusted for body weight or body surface area (BSA). Three-dimensional configurations of the soft palate were evaluated using medical viewing software, OsiriX 7.0 (Pixmo, Bernex, Switzerland). The grading of the morphological features of the soft palate was performed for all brachycephalic and non-brachycephalic breeds. The first author and other authors (Y.J.K., N.R.L., J.Y., H.B.L., G.Y.A., and S.H.B.) had discussed and reached a consensus in order to determine the grade of soft palate. A Grade 1 soft palate was defined as one with a sharp, plate-like shape, Grade 2 as a dull, club-like shape, and Grade 3 as an irregular, club-like shape. Three images were extracted from one dog, then obliquely rotated so as to help in understanding three-dimensional structure (Fig. 3).

**Statistical analysis**

A Kolmogorov-Smirnov test confirmed the assumption of normal distribution for all parameters. All two and three-dimensional parameters were summarized as mean and standard deviation (SD) and compared between breeds using an independent samples \( t \)-test. Pearson linear correlation was performed between the volume of nasopharynx and (1) soft palate to skull ratio, (2) maximum thickness of soft palate, and (3) body weight in all 127 dogs. The correlation between body weight and the volume of soft palate was also evaluated. Variables were added to the multivariable model in a manual stepwise manner, initially including all variables that were statistically significant in the univariate analysis, and then excluding those not reaching statistical significance one by
one, until all the variables included showed a statistical significance. Multiple linear regression analyses were performed for the overall population. Variables assessed for their effect on outcome in each group were soft palate to skull ratio, maximum thickness of soft palate, soft palate volume, and nasopharyngeal volume. All variables were adjusted for BSA.

Data analysis was performed with the aid of a standard statistical software package (SPSS 11.0 for Windows, SPSS Inc., Chicago, IL, U.S.A. and Prism 6.0, Graph-pad Software, San Diego, CA, U.S.A.). Values of $P<0.05$ were considered significant for all tests.

RESULTS

Signalment

A total of 127 dogs were included in this retrospective study. These included 42 brachycephalic breed dogs, 85 non-
Brachycephalic breed dogs. Brachycephalic breeds included Shih Tzu (n=19), Pekingese (n=3), Pomeranian (n=10), Chihuahua (n=8), Cavalier King Charles Spaniel (n=1), and Pug (n=1) and non-brachycephalic breeds included Poodle (n=12), Bichon Frise (n=1), Spitz (n=3), Miniature Schnauzer (n=8), Dachshund (n=3), Maltese (n=37), Yorkshire Terrier (n=12) and mixed breeds (n=9). Brachycephalic dogs had a mean age of 6.6 ± 0.73 years and a mean body weight of 4.9 ± 0.17 kg, non-brachycephalic dogs had a mean age of 7.4 ± 0.91 years and a mean body weight of 4.6 ± 0.34 kg. There was no significant difference in age (P=0.296) and body weight (P=0.488) between the brachycephalic and non-brachycephalic dog breeds (Table 1). Sex was tested by Pearson’s χ² test and also did not show a significant difference (P=0.578) between the two groups.

Quantitative Assessment of MR Images

There were consistent, statistically different in skull length (P<0.01), soft palate to skull length ratio (P<0.01) and maximum thickness of soft palate (P<0.01) between the brachycephalic and non-brachycephalic dog breeds after body weight normalization (Table 1), but not for the length of the soft palate. The skull length was significantly shorter and maximum thickness of soft palate was much greater in brachycephalic breed when normalized for body weight. There was no significant difference between the two breeds in the length of soft palate prior to being adjusted to skull length (P=0.369) (Table 1). However, the soft palate length adjusted by skull length, soft palate to skull length ratio, was significantly higher in brachycephalic breeds. Mid-sagittal nasopharyngeal airway CSA was smaller in the brachycephalic breeds (P<0.01) after normalization for body weight, while the CSA of soft palate was much larger (P<0.01) (Table 1). The nasopharyngeal volume of brachycephalic dog breeds was significantly smaller in comparison with the non-brachycephalic dog breeds (P<0.01) (Table 1). There was no significant difference

Table 1. Age, BW and BSA for brachycephalic breeds and non-brachycephalic breeds are presented. Following two-dimensional and three-dimensional parameters are presented

| Parameters                  | BC          | NBC         | P-value |
|-----------------------------|-------------|-------------|---------|
| Age (years)                 | 6.61 ± 0.73 | 7.4 ± 0.91  |         |
| BW (kg)                     | 4.97 ± 0.17 | 4.6 ± 0.34  |         |
| BSA (m²)                    | 0.29 ± 0.007| 0.27 ± 0.008|         |
| 2D parameters               |             |             |         |
| SL/BW (cm/kg)               | 2.05 (0.45) | 2.65 (0.55) | <0.01   |
| SPL/BW (cm/kg)              | 0.83 (0.27) | 0.88 (0.23) | 0.369   |
| SP/SL ratio                 | 0.40 (0.09) | 0.33 (0.06) | <0.01   |
| SPT/BW (cm/kg)              | 0.15 (0.04) | 0.10 (0.03) | <0.01   |
| SP CSA/BW (cm²/kg)          | 0.31 (0.15) | 0.13 (0.04) | <0.01   |
| NP CSA/BW (cm²/kg)          | 0.30 (0.18) | 0.55 (0.17) | <0.01   |
| 3D parameters               |             |             |         |
| SPV/BW (cm³/kg)             | 0.39 (0.14) | 0.41 (0.11) | 0.585   |
| NPV/BW (cm³/kg)             | 0.22 (0.15) | 0.48 (0.19) | <0.01   |
| SPV/Total ratio             | 0.65 (0.13) | 0.46 (0.08) | <0.01   |
| NPV/Total ratio             | 0.34 (0.13) | 0.53 (0.08) | <0.01   |
| SP Grade (%)                |             |             |         |
| I                           | 19          | 85          |         |
| II                          | 48          | 15          |         |
| III                         | 33          | 0           |         |

Mean (standard deviation). There was no significant difference in age and body weight between the brachycephalic and non-brachycephalic dog breeds. BW, Body Weight; BSA, Body Surface Area; SL, Skull Length; SPL, Length of Soft Palate; SP/SL, Soft Palate to Skull Length ratio; SPT, Maximum Thickness of Soft Palate; SP CSA, Cross-Sectional Area of Soft Palate; NP CSA, Cross-Sectional Area of Nasopharynx; SPV, Soft Palate Volume; NPV, Nasopharynx Volume.

Fig. 3. Representative examples of Grade of Three-dimensional Reformatted Configuration of Soft Palate. The each set of three examples elicited from the same objects and were obliquely rotated to help in understanding three-dimensional structure of the soft palate. (A) Grade 1: Sharp, Plate-like shape. (B) Grade 2: Dull, Club-like shape. (C) Grade 3: Irregular, and Club-like shape. Three-dimensional configurations of soft palate were evaluated using medical viewing software, OsiriX 7.0 (Pixmo, Bernex, Switzerland).
in the volume of soft palate between the two groups until being normalized by skull length. When the volume was adjusted for skull length, the difference became significant ($P<0.01$). The ratio of the soft palate to the total volume, which is the sum of the nasopharynx and soft palate, was significantly greater in brachycephalic breeds ($P<0.01$), which the nasopharyngeal volume-total volume ratio was significantly smaller ($P<0.01$).

There was significant correlation between the nasopharyngeal volume and the maximum thickness of soft palate ($r=−0.424$, $P<0.01$) as well as body weight ($r=−0.295$, $P<0.01$) (Fig. 4). Body weight did not correlate with volume of soft palate ($r=−0.070$, $P=0.434$) (D).

The percentage of each grade of three-dimensional configuration of the soft palate in brachycephalic breeds was 19% in grade1, 48% in grade2 and 33% in grade3; in non-brachycephalic breeds, it was 85, 15, and 0%, respectively (Table 1). Fisher’s exact test showed significant difference in $P<0.01$ and brachycephalic breeds showed higher grade of three-dimensional configuration.

When all factors were inserted into a stepwise multiple regression analysis model, the factors determining three-dimensional configuration of soft palate included ratio of the volume of the soft palate ($P<0.01$), nasopharyngeal volume ($P<0.01$) and the maximum thickness of soft palate ($P<0.01$) to body surface area (Table 2). This model accounted for 44.6% variance ($r=0.667$, $P<0.01$). Given the variance inflation factor (VIF) values of all the factors were less than 10, there was no multicollinearity among the factors.

**DISCUSSION**

This comparative description of the upper airway morphology of brachycephalic and non-brachycephalic dogs using MRI showed many distinctive features. This is the first study of brachycephalic breeds to use MRI to delineate the airway and the tissues surrounding the airway in detail.

Abnormal under-development of the maxilla in proportion to the body size is the key characteristic of brachycephalic dog breeds...
In this study, the skull length was significantly shorter in brachycephalic breeds and this corresponds well with the previous knowledge of the anatomy of these breeds. However, we could not find a significant difference in the length of the soft palate between brachycephalic and non-brachycephalic dogs. We think that this result was derived from the difference in the absolute length of the skull between the two groups. In non-brachycephalic dog breeds, the skull length was much longer than that in brachycephalic dogs, so the length of the soft palate was long as well; when the length of the soft palate was adjusted according to skull length, the difference between the breeds reached statistical significance (P=0.005). This result showed that the relative length of the soft palate is much longer in brachycephalic breeds. Elongated soft palate is well known feature of brachycephalic breeds and this also corresponds with the results of this study. The maximum thickness of the soft palate on the midline sagittal MRI was significantly greater in brachycephalic breeds. A thickening of the soft palate has recently been proposed as a component in severe brachycephalic airway syndrome and our study supports this theory [10]. The CSA of the soft palate measured in the mid-sagittal image was significantly larger, and the CSA of nasopharynx was significantly smaller in brachycephalic breeds. The narrowed dimension of the nasopharynx is considered as an acceptable surrogate measure of the severity of the nasopharyngeal conformational anomaly. One study showed that the CSA of the nasopharynx measured in transverse image at the level of the rostral border of the tympanic bulla was smaller in the brachycephalic dogs with bilateral otitis media than dogs without the disease [11]. Our study, however, showed no significant difference in the dimension measured in transverse images. We think that this is because the severity of the clinical presentation was not taken into consideration in defining the population.

The volume of the soft palate showed no difference between the two groups. This result was also affected by the absolute length of the skull as the same reason in the results of comparison of the soft palate; when the volume was adjusted for skull length, the difference was significant difference (P<0.01). This means that the relative volume of the soft palate is much greater in brachycephalic breeds. This is the comparable to studies in the human medicine on OSAS patients. One study showed that the volume of the soft palate was about 30% greater in subjects with OSAS [1]. The large soft palate volume might be the one of the main factor in brachycephalic airway syndrome, as in OSAS patients. The reason why brachycephalic dogs usually have enlarged width and thickened soft palate is revealed in several studies. It could be explained by muscular hypertrophy of the palatal muscle, mucosa edema, or both.

The volume of the nasopharynx is significantly smaller in brachycephalic breeds. The pathophysiology of pharyngeal collapse has not been completely elucidated, but it is likely multifactorial in nature. Pharyngeal anatomy and pharyngeal dilator muscle responsiveness are probable important components [6]. In brachycephalic dogs, chronic negative pressure gradients with increased inspiratory upper airway resistance may cause pathologic changes to develop in the pharyngeal dilator muscles and therefore predispose to pharyngeal collapse. Pharyngeal collapse is well described in humans and is thought to be the major component of OSAS [4]. The significantly decreased pharyngeal volume in brachycephalic dogs means that pharyngeal collapse could be the major factor for brachycephalic airway syndrome as in OSAS.

In our study, the nasopharyngeal volume was significantly correlated with the maximum thickness of soft palate and body weight (Fig. 4). Body weight correlated with smaller nasopharyngeal volume and larger soft palate volume. Obesity is reported to be the main factor in increasing the size of the soft-tissue structures, decreasing the size of the upper airway [20]. However, in our study, we could not conclude the body condition score as a factor attributed to the fact that it was not available. Since absolute body weight might not be the right surrogate for obesity, our study cannot determine the effect of obesity to the result. An elongated soft palate has been accepted as contributing to the brachycephalic airway syndrome for a long time, but more recently the importance of an abnormally thick soft palate has also been recognized [9, 10]. In humans, craniofacial morphology influences air-flow and the apnea-hypopnea index, such that these individuals have an increased risk of OSAS [3, 21]. Although such a scoring system has not been established in dogs, given that nasal pressure is the key semi-quantitative measure of airflow in human, craniofacial morphology of brachycephalic breeds may be a strong contributory factor to pharyngeal collapse.

The grade of three-dimensional configuration of the soft palate was significantly different between the two groups. Most of the non-brachycephalic breed dogs were included in grade 1; sharp and plate-like shape. In brachycephalic breed dogs, grade 2 was the common shape of the soft palate. The shape alteration to grade 2; dull and club-like shape, was mainly derived from the thickened and broadened soft palate. The change in soft palate shape to grade 3; irregular and club-like shape, might be more complicated. Chronic negative nasal pressure and narrowed upper airway can result in secondary morphological changes of the soft palate. Recent histological findings have shown that the thickness of soft palate was not due to muscle hypertrophy or fat deposition [5]. Histological evaluation of the soft palate of brachycephalic dogs showed that the most important microscopic findings were intracellular edema of mucosal lining, diffuse edema, and amplified myxoid matrix in the lamina propria. It is believed that mechanical trauma like snoring and stridor elicited by obstructive upper airway can produce such changes [17]. The major factors affecting the morphology of soft palate were soft palate volume, nasopharyngeal volume, and thickness of soft palate. This suggests that the morphological changes in the soft palate are affected more by the volume or thickness, and less by length. As the change in shape of the soft palate is secondary to chronic negative pressure of upper airway, the decreasing nasopharyngeal volume, increasing soft palate volume and three-dimensional morphological change of soft palate are all related and occur at the same time.

The major limitations of this study are associated with its retrospective nature. There was limited scope in obtaining images; the laryngeal area could not be evaluated. Obesity, which has been identified as the main risk factor for pharyngeal collapse and brachycephalic airway syndrome, could not be taken into consideration. Additionally, because anesthesia is necessary for MRI scans in dogs, all images were obtained under anesthesia. Anesthesia can influence the muscular tone of the pharyngeal area, especially the muscular tone of the soft palate, and could have influenced the measurements. Lastly, we did not exclude the dogs with brachycephalic airway syndrome. Hence, there could be possible that the morphologic change described in this study between
brachycephalic dogs and non-brachycephalic dogs could arise from brachycephalic airway syndrome, not the breed itself.

In conclusion, we discovered a significant difference in two-dimensional and three-dimensional parameters between brachycephalic and non-brachycephalic dog breeds using MRI. The increased thickness and volume of the soft palate are the significantly relevant parameters for narrowing of the nasopharynx. These morphological changes of the soft palate partially contribute to nasopharyngeal collapse. The chronic changes in craniofacial morphology of brachycephalic breeds exacerbate overcrowding of the upper airway and further elevate upper airway pressure; this might be related to severe brachycephalic airway syndrome. This study demonstrates the possibility of using MRI to evaluate brachycephalic airway syndrome, as is done for OSAS in humans.

Further studies are warranted to prospectively investigate whether the anatomical differences in upper airway or transformation of the soft plate appearance exist among brachycephalic dogs depending on the presence of clinical signs of brachycephalic airway syndrome.

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