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Factors Correlated with Post-Orthodontic Treatment Gingival Enlargement

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ABSTRACT

Introduction: It has been established that gingival inflammation is exacerbated during puberty, in women taking oral contraceptives, and during pregnancy. Increased levels of sex hormones are implicated in the changes in periodontal conditions. It has also been established that treatment with fixed orthodontic appliances presents a favorable situation for plaque accumulation, making oral hygiene more difficult to maintain. Therefore, it was hypothesized that adolescents receiving orthodontic therapy during their pubertal growth peak, might be more susceptible to gingival enlargement than those being treated outside of puberty. Orthodontists often seek to treat adolescent patients during their pubertal growth peak and utilize the Cervical Vertebral Maturation (CVM) Index proposed by Baccetti et al in 2005 to identify this time point. The purpose of this study was to evaluate whether orthodontic patients treated during their pubertal growth spurt, defined as cervical stage 3 – cervical stage 4 by the CVM index, are more susceptible to gingival enlargement than those treated outside of puberty. Additional factors, such as gender, race, treatment length, and initial spacing, might be correlated with the severity of gingival enlargement. Therefore, these conditions were evaluated and controlled for.

Methods: A retrospective review of the records of 232 patients treated with comprehensive orthodontics between the ages of 10 and 25 years old at the Medical University of South Carolina was performed. Subjects' pre-treatment intraoral photographs were evaluated from canine-canine for hygiene and amount of spacing. The

final cephalograms were judged for cervical stage to indicate proximity to the pubertal growth spurt. A new method for photographically assessing gingival enlargement is proposed as part of this research and was utilized to evaluate the gingival condition in the final intraoral photographs. Statistical analysis was performed to identify factors correlated with post-orthodontic gingival enlargement, specifically: gender, ethnicity, initial hygiene, length of treatment, treatment type (space closure vs. crowding relief), and proximity to the pubertal growth spurt.

Results: No correlation was found between gender, ethnicity, length of treatment, or pubertal stage and severity of gingival enlargement. Initial hygiene, amount of space, and arch (upper vs. lower) were associated with the severity of gingival enlargement. Patients with worse initial hygiene and more initial spacing exhibited greater degrees of gingival enlargement. More severe gingival enlargement was also found in the lower arch than the upper arch.

Conclusions: Severity of photographically-assessed post-orthodontic gingival enlargement does not appear to be correlated with proximity to the pubertal growth spurt. Patients with poor initial hygiene and those planned for closure of anterior spacing are at risk for increased levels of gingival enlargement, especially in the lower arch. Increased efforts to manage hygiene during orthodontic treatment might be warranted in such patients.

REVIEW OF LITERATURE

Sex Steroid Hormones and the Periodontium

Since the 1960s, investigators have been examining the influence sex hormones have on the periodontal tissues. More than a century's worth of clinical observations of exaggerated gingival inflammation during puberty, pregnancy, and oral contraceptive use, when sex steroid hormone levels are high, triggered a profusion of research. Potent steroid hormones are certainly worthy of such investigative attention. The primary sex steroid hormones, androgens, estrogens, and progestins, are involved in the regulation of diverse tissues beyond the reproductive system, such as the brain, heart, kidney, skin, and liver.[1]

Testosterone and androstenedione, the principal plasma androgens in men and women respectively, are synthesized in the testes, ovaries, and adrenal cortex. Testosterone is irreversibly reduced to dihydrotestosterone, which is responsible for most actions of the hormone.[1] With male sexual differentiation being one of the primary actions of testosterone, the hormone's levels rise sharply during puberty. Estradiol is the most potent and abundant estrogen in women and is secreted by the ovaries, testes, placenta, and some peripheral tissues.[1] Like testosterone, estradiol plays a key role in sexual differentiation and thus rises dramatically during puberty. In addition, estradiol levels rise cyclically following menarche and remain high should fertilization and implantation of the embryo occur. The third and final category of steroid sex hormones, the progestins, work in concert with the estrogens, and rise after ovulation during the

menstrual cycle and remain high during pregnancy. Progesterone, the main progestin, is secreted by the corpus luteum, the placenta, and the adrenal cortex.

The specific responses illicited by androgens, estrogens, and progestins depend on the presence of receptors in the various hormone-sensitive target tissues. Free hormones can diffuse through the cell membrane and bind to intracellular protein receptors in both the cytoplasm and nucleus. Once inside the nucleus, the receptor-hormone complex can bind to DNA sequences and activate gene transcription. It was previously thought that this was the primary mechanism of action for sex steroids, but recent studies have shown that androgens, estrogens, and progestins have membrane effects as well; they can affect neural transmission and modify the transport of calcium ions into cells.[1] Many studies have sought to evaluate the periodontium as a potential hormone-sensitive target tissue. Autoradiographic studies have demonstrated nuclear localization of estradiol and a synthetic androgen (methyltrienolone) in human gingival epithelium and fibroblast cells. However, accumulations of progesterone have not yet been demonstrated in gingival epithelial cells.[1] Intracellular receptors for estrogens, androgens, and progesterone have been partially characterized in the periodontium. In fact, the number of estrogen and androgen receptors in the gingiva increases almost tenfold during gingival inflammation.[2,3] The data support the categorization of the periodontium as a target tissue for sex steroid hormones.

Much research effort has been applied to identifying what effects the steroid sex hormones might have once activated in the periodontal target tissue. Throughout the body, estrogen can have powerful effects on blood flow. Estrogen stimulates blood flow

in the uterus and endometrial flow during the menstrual cycle. It can also increase the movement of fluid across blood vessel walls within minutes of administration.[4] Similarly, testosterone, once metabolized to estradiol in males, can dilate blood vessels in accessory sex organs.[5] The effects of progesterone on blood vessels are debatable. Studies by Lindhe and Branemark in 1967 concluded that progesterone increased vascular permeability and proliferation.[6] However, Mariotti[1] points out that the doses of ovarian hormones studied were up to 400,000,000 times the plasma concentration found in non-pregnant human females (1,000,000 times those in pregnant females) and that the effects on hamster cheeks and rabbit ears may not be transferrable. Regardless of the specific hormone at play, it has been demonstrated that gingival vasculature is responsive to the sex steroid hormones. The amount of gingival crevicular fluid (GCF) is related to the permeability of the dentogingival vessels. Elevated GCF levels have been demonstrated in the presence of sex steroid hormones and is as much as 54% higher in pregnant females compared to postpartum controls.[7] Finally, Hugoson and Lindhe[8] found that in inflamed and inflammation-free canine dentitions, exogenously administered estrogens and/or progestins significantly increased the amount of GCF. At the cellular level, most of the research on androgens, estrogens, and progestins has focused on gingival keratinocytes and fibroblasts. Estrogens have been found to stimulate epithelial proliferation and increase keratinization. Androgens have also been found to increase epithelial cell count. One study ascertained a reduction in keratinization of human gingival cells after progestin administration, but the authors posit that the results might be more due to a reduction of plasma estradiol induced by daily progesterone

administration.[9] Current research on fibroblasts indicates that androgens and progestins have an inhibitory effect, but estrogens appear to be stimulatory.[1] The progesterone and estrogen effects on fibroblasts are reported to be quite significant in humans, with 40 μg/ml of progesterone reducing protein synthesis up to 50%, and 1η*M* of estradiol stimulating cell proliferation from 50% to 310%.[1,10] In addition to increasing fibroblast proliferation, estrogens appear to be involved in the synthesis and maintenance of fibrous collagen, but more conclusive research is needed.[1,11] Mariotti summarizes the sex steroid cellular research best.

> There is evidence to suggest that gonadal hormones mediate the actions of some gingival fibroblasts and epithelial cells and therefore contribute to the maintenance of this tissue. It is known that gingival tissues and/or cells metabolize sex steroid hormones, contain hormone receptors, and proliferate in the presence of specific steroids... Despite the observed influence of sex steroid hormones on the gingiva, the specific effects of gonadal hormones on cellular function in this tissue remain to be elucidated.[1]

Puberty Gingivitis and Hormone Levels

The increased levels of sex steroid hormones during puberty, pregnaney, and oral contraceptive use and their established potential cellular effects has made them primary suspects in the etiology of the increased incidence of gingival inflammation observed during these phases. For the purposes of this study, we were most interested in hormonal impacts during puberty. Two studies have examined the ages at which circumpubertal gingival inflammation peaked. Sutcliffe[12] followed 127 children over 6 years and documented a sudden, yet transitory, increase in the incidence of gingivitis without a corresponding increase in plaque. The peak prevalence of gingivitis was found at 12 years, 10 months in females, and 13 years, 7 months in males. In a cross-sectional study

of 7,380 children, written in the Swiss language, Hefti et al[1,13] found an increase in gingival inflammation at 11 years old in both sexes despite constant plaque levels. In both studies, the ages at which gingival inflammation peaked corresponded with the onset of puberty. However, because chronologic age is a poor indicator of pubertal status, these data can only be considered circumstantial evidence that pubertal hormones contribute to gingival inflammation.[1] Morishita et al[14] attempted to address this issue by studying the salivary concentrations of estradiol, progesterone, and testosterone in 1,323 schoolchildren 12-15 years old. Salivary concentrations of sex hormones have been reported to exhibit a strong positive correlation with those in serum.[15,16] Morishita's[14] results indicate that "(1) males with a greater tendency toward bleeding on probing had high salivary levels of estradiol, (2) males and females with low pocket depth values or low subgingival counts had high salivary progesterone, and (3) testosterone level did not have any relation to G.I., P.D., bleeding, or subgingival bacterial counts." In addition to increased levels of salivary sex hormones during puberty, subgingival microflora counts increase and change in composition. Studies have shown an increase in *Prevotella intermedia*, and *Capnocytophaga* species during puberty, and *Actinomyces viscosus* and *Eikenella corodens* in patients with diagnosed puberty gingivitis.[17,18] Kornman and Loesche[19] found that *Prevotella intermedia* has the ability to substitute estrogen and progesterone for vitamin K as an essential growth factor. The research around pubertal gingival inflammation implicates sex hormones in the exacerbated response to dental plaque.

Adolescent Hygiene and Hygiene during Orthodontic Treatment

Much of the research evaluating pubertal hormones associates the aggravated gingival response to dental plaque to the *female* sex hormones. Fortunately, healthy women experience minimal and transient side effects from various hormone levels and females have demonstrated a better oral condition than males.[11] In fact, adolescent males are repeatedly documented to have worse hygiene habits than their female counterparts. In one longitudinal study comparing adolescents at 11-12 and at 15-16 years old, controlling for class and toothbrushing frequency, boys consistently had higher plaque, bleeding, and pocketing scores than girls. Addy[20] concludes that "the results demonstrate the influence of ... sex rather than toothbrushing frequency... on oral hygiene and gingival health." In reporting the findings from the National Survey of Oral Health in US Schoolchildren conducted during 1986-1987 on 14-17-year-old children, Bhat[21] noted that the boys consistently had a higher proportion of gingival bleeding on probing and approximately 3% higher prevalence rates for supra- and subgingival calculus. As Amar and Chung[11] note, a lack of correlation between puberty and periodontal clinical parameters in girls might be explained by their superior oral hygiene habits.[11]

Zachrisson[22] points out that the addition of fixed orthodontic appliances into the oral cavity increases the number of retentive areas, thus creating a more plaque-friendly environment and compounding the risk of gingivitis in adolescents with a poor hygiene tendency. Enlarged gingival tissues make access to the tooth surface difficult, exacerbating an already inflammatory situation. Indeed, gingivitis is found in most orthodontically treated patients, even those with impeccable hygiene.[22] A longitudinal study of gingival changes during the full period of orthodontic treatment was performed

by Zachrisson[22] in1972. Forty-nine adolescents from 11-13 years old at the beginning of treatment were followed and compared to a non-orthodontically treated control group. Plaque accumulation, gingivitis, and gingival hyperplasia were recorded before, during, and at multiple points after treatment. Several key conclusions were made by Zachrisson:

1. Most children developed moderate generalized gingivitis during orthodontic treatment.

2. The gingival changes occurred within one to two months after orthodontic appliance placement.

3. The plaque and gingivitis scores were higher at the time of appliance removal than at any other time.

4. Inflammatory changes were evident, especially interproximally, even in patients with excellent hygiene.

5. Gingival hyperplasia was higher interproximally than at the buccal surfaces.

6. Increased pocket depths during treatment were due to edematous swelling and tissue accumulation rather than apical attachment loss.

7. The gingival health improved rapidly within the first month after appliance removal.

8. The gingival changes were transient and no permanent damage to the periodontal tissues was noted.

With two recent exceptions, these results have been corroborated by subsequent studies.[23,24] Recently, Kouraki et al[25] and Gong et al[26] found that gingival enlargement was not completely reversible upon removal of orthodontic appliances. Kouraki et al propose that the difference in their findings may be due to the variety of techniques used in assessing gingival enlargement or the different levels of hygiene control between the groups studied. Interestingly, Kouraki's results did not show a correlation between plaque score or gingival index and the degree of gingival enlargement. As far as the

induction and resolution of gingival enlargement, Gong et al[26] investigated the associated microbiologic and immunologic factors. Subgingival plaque and gingival crevicular fluid samples were collected from gingival enlargement sites in 12-18 year olds about 9.5 months into orthodontic treatment and again four weeks after appliance removal and periodontal therapy at the involved sites. They found that the levels of *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, *Treponema denticola*, and *Tannerella forsythia*, all periodontal pathogens, were significantly higher at sites with gingival enlargement than at the control sites. They also found that inflammatory cytokine (IL-1β and TGF-β1) levels at the gingival enlargement sites were significantly higher than those at the control sites. Four weeks after periodontal therapy, 2 of the 12 patients studied still had severe gingival enlargement. Gong et al agree with Kouraki et al that complete resolution of orthodontic treatment-induced gingival enlargement is not always attained. Once gingival enlargement is established, it appears that improved hygiene and reduced plaque levels do not seem to improve the gingival condition. These investigators suggest that possible fibrotic changes take place that prevent the gingiva's ability to return to health even after removal of irritants (plaque/orthodontic appliances). Perhaps this is where hormonal variations play a role, especially considering that Kouraki et al's and Gong et al's subjects were between 11-18 years old at the start of orthodontic treatment.

Evaluating Gingival Enlargement

In their discussion, Kouraki et al[25] mentioned the differences in technique when evaluating gingival enlargement. A variety of methods, primarily developed by investigators studying the side effects of drug-associated gingival enlargement, can be found throughout the literature. In 1972, Angelopoulos and Goaz[27] developed the hyperplastic index (HI) that was further modified by Pernu et al[28] in 1992 (Fig 1). The HI defines four defined categories of gingival enlargement: 0, no gingival overgrowth; 1, mild overgrowth, blunting of the marginal gingival; 2, moderate overgrowth, extending to the middle of the tooth crown; and 3, severe overgrowth, covering two thirds of the tooth crown or affecting the whole of the attached gingiva. For this investigator, the HI proved to be too ambiguous to allow for an acceptable intra-examiner error when applied to intraoral photographs. Most of this investigator's error came from distinguishing 0s from 1s, and 2s from 3s. The developers of the HI did not specify if "middle" and "two thirds" of the tooth surface was when measuring mesio-distally or incisal-gingivally. It also did not appear that the example photos reflected their categorization. How can the gingiva cover two thirds of the tooth crown if a bracket is present? Seymour et al[29] developed a method of quantifying gingival enlargement on plaster study models using both the thickness and the height of the gingival tissues (Fig 2). In this method gingival thickening is graded as: 0, normal; 1, thickening from the normal up to 2mm; and 2, thickening from the normal greater than 2mm. The height of the gingival tissue is graded as 0,1,2,3. The two scores are added for each site, so a maximum obtainable score for each site is five. Although Kouraki claimed to calibrate himself to be able to use this method effectively, this investigator questions the degree of error when measuring 2mm

increments from "normal" for gingival thickness on plaster models. In addition, the gingival height gradations are more subjective than quantifiable. Although the Seymour method has potential on plaster models, this investigator was unable to transfer the gingival thickness evaluation to intraoral photographs taken from the facial and buccal. Therefore, as part of this research, a variation on the above methods is applied to evaluate gingival enlargement from intraoral photographs.

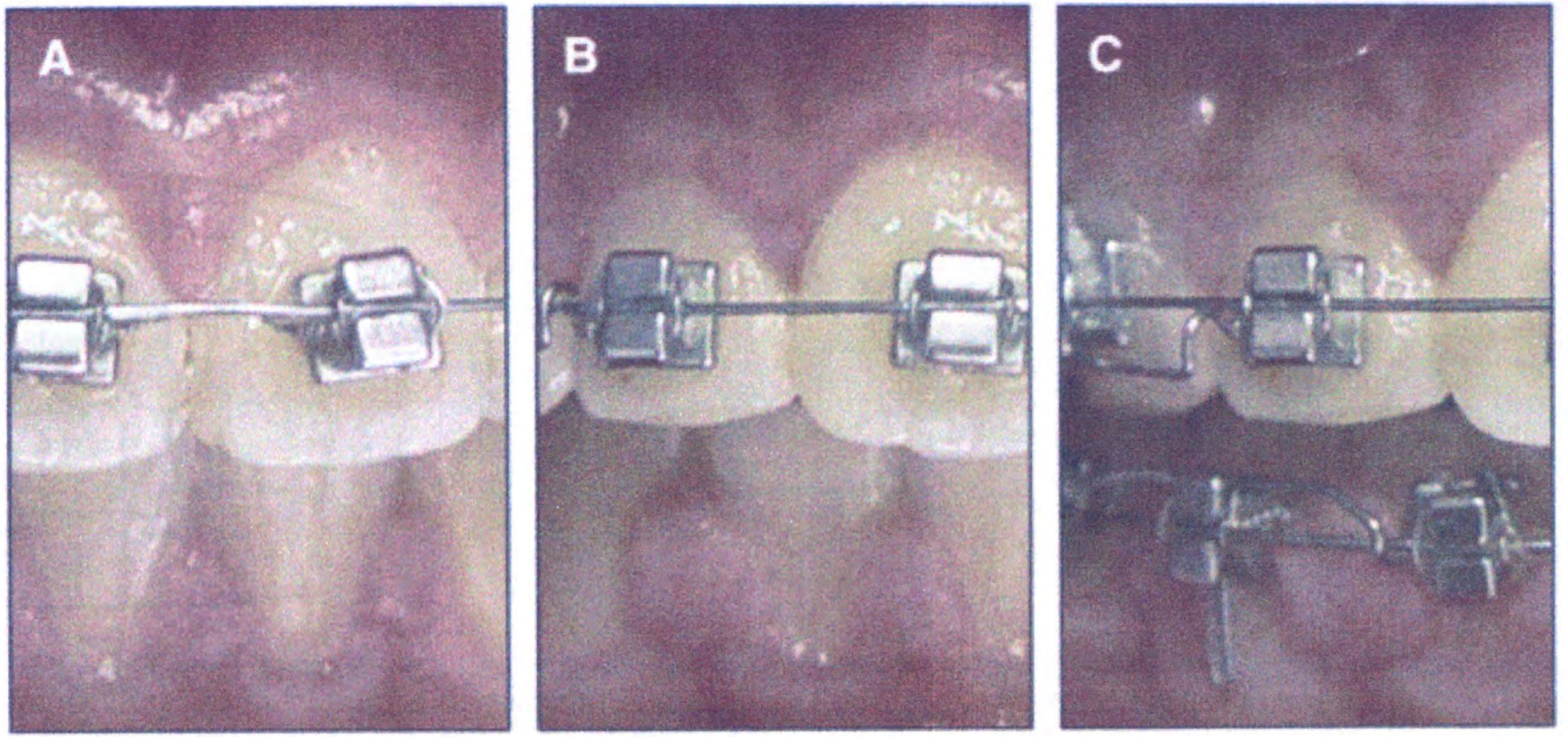


Fig 1. Hyperplastic Index assessment of mandibular gingiva: **A**, score 1: mild overgrowth; **B**, score 2: moderate overgrowth; **C**, score 3: severe overgrowth.[27]

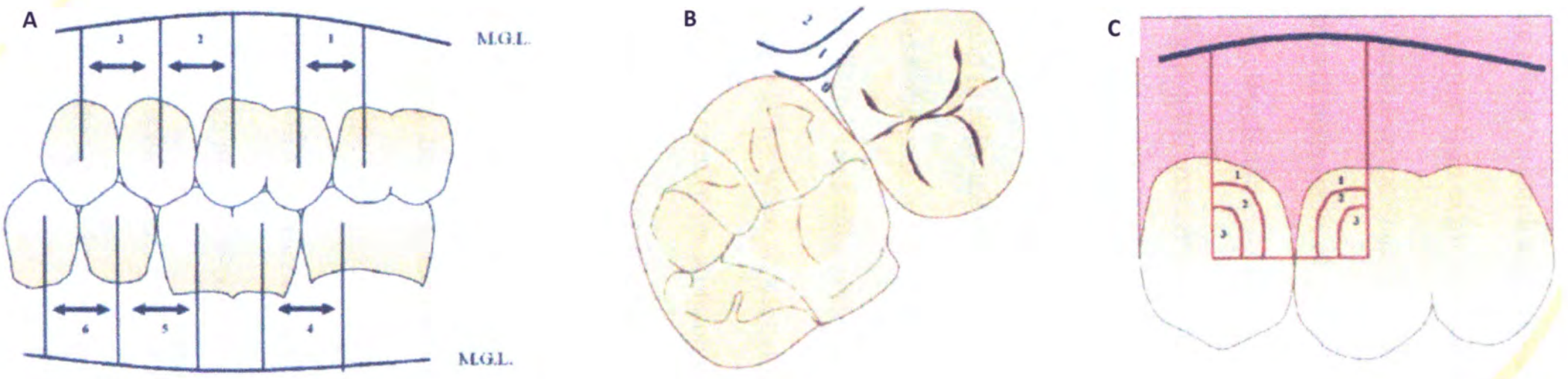


Fig 2. Seymour Gingival Enlargement assessment: **A**, example of how segments to be evaluated are defined; **B**, Degree of gingival thickness, graded as 0, 1, or 2; **C**, height of gingival tissue graded as 0,1,2,or 3.[29]

Evaluating Pubertal Stage

A more robust research and clinical history exists for evaluating proximity to the pubertal growth spurt than exists for evaluating gingival enlargement. Growth potential is an essential input into the diagnosis and treatment planning of the orthodontic patient. Mandibular growth potential is of primary importance to the timing and treatment effectiveness of class II and class III skeletal patterns. For decades, orthodontists have been evaluating skeletal maturation radiographically. In 1959, Greulich and Pyle[30] published an atlas of hand-wrist radiographs that illustrated multiple ossification events correlated to an individual's skeletal maturity. The peak growth velocity in statural height was found to coincide with the appearance of the adductor sesamoid bone of the thumb. For most, the peak in mandibular growth occurs at the same time as the peak in statural growth.[31] The hand-wrist radiograph was the standard in orthodontics for many years until the late 1970s when an attempt was made to identify a skeletal maturation indicator that did not require the additional radiograph. The cervical vertebrae, which are visible on the lateral cephalogram taken as a standard part of pre-treatment orthodontic records, were studied for potential maturational indices and correlation with mandibular growth changes.[32-37] Currently, the most widely-used cervical vertebral indices are those developed by Franchi, Baccetti, and McNamara in 2000 and refined in 2005.[33] These investigators studied the archived annual cephalometric files of thirty subjects collected in the University of Michigan Elementary and Secondary Growth Study conducted from the mid-1930s through the late 1960s. The maximum increase in mandibular length (as measured from the cephalometric points Condylion to Gnathion) defined the pubertal growth spurt. Morphological characteristics of the cervical vertebrae were evaluated at

the two consecutive cephalograms contiguous to the pubertal growth spurt, as well as the two previous and two subsequent annual radiographs. Six stages of cervical vertebral maturation (CVM) were developed, with the peak in mandibular growth occurring between cervical stage (CS) 3 and cervical stage 4. Critical to the applicability of CVM method, as with any method, are its reproducibility and accuracy. In a recent systematic review of accuracy and reproducibility studies on current CVM methods, Santiago et al[38] concluded that the studies "suffer from severe methodological failures" and that better designed studies are needed before these methods can be confidently applied. Indeed, only two studies on the 2005 Baccetti et al[33] method met their moderate to high quality rating: one study by Lai et al[39] and one by Gabriel et al[40]. The Lai study concludes that the 2005 Baccetti CVM method is accurate when compared to the hand-wrist standard. The Gabriel study, however, found poor inter- and intra-examiner reproducibility. Until the accuracy and reproducibility issues are resolved in the scientific literature, the 2005 Baccetti CVM method remains the current norm. For the purposes of this research, this CVM method was applied, but not until a high level intra-examiner reproducibility was reached.

Gingival Enlargement and Space Closure

When studying orthodontically-associated gingival enlargement, the potentially multi-factorial nature of the condition cannot be overlooked. Naturally, gender and race differences should be evaluated. In addition, hygiene must be controlled for due to the inflammatory component of gingival enlargement. And for the focus of this study, proximity to the pubertal growth spurt was of upmost importance. A less obvious influencing factor is space closure. Gingival hyperplasia is an expected side effect of

orthodontic closure of an extraction site.[41] As the teeth are moved together, an invagination of epithelium and connective tissue is formed. Most of the observations of gingival overgrowth associated with space closure are noted with closure of premolar extraction sites as opposed to closure of generalized spacing between the teeth. The average premolar measures 7mm in mesio-distal width, thereby leaving a 7mm space to close after extraction. Anterior spacing, which is the focus of this research, is rarely at the level of 7mm. However, whether significant space was closed or crowding was relieved during orthodontic treatment, could impact the degree of gingival enlargement observed.

In summary, the purpose of this research was to investigate descriptive factors that might be correlated with the severity of gingival overgrowth during orthodontic treatment, specifically gender, race, pre-treatment hygiene, length of treatment, type of treatment (space closure vs. crowding relief), and proximity to the pubertal growth spurt. We expected to find that females treated around their pubertal growth peak have more significant gingival overgrowth than their male counterparts when controlling for hygiene. We also expected to find more significant gingival enlargement in space closure cases but expected no impact of race or treatment length.

MATERIALS AND METHODS

The materials and methods implemented in this study were approved by the Institutional Review Board of the Medical University of South Carolina.

Study Subjects

Resource:

The records of all orthodontic patients treated with comprehensive orthodontics at the Medical University of South Carolina were reviewed.

Inclusion Criteria:

All male and female patients whose treatment was completed between 11 and 25 years of age were included.

Exclusion Criteria:

The following subjects were excluded:

- Subjects whose pre-treatment records were gathered more than six months prior to the initiation of orthodontic treatment
- Subjects with craniofacial syndromes or mental disabilities.
- Subjects in whom not all upper and lower incisors were erupted in the pre-treatment records.
- Subjects missing any of the upper or lower anterior teeth (canine-canine).

Demographics:

In total, 232 records were included with the following age, gender, and ethnic breakdown. Figures 3 and 4 and Tables I and II breakdown the ethnicity and ages of the subjects studied.

Methodology

For each subject, four values were assessed: treatment type (space closure vs. crowding relief), pre-treatement hygiene, cervical vertebral maturation stage, and final gingival enlargement. One investigator performed the evaluation of these variables according to the methods described below.

Pre-treatment Hygiene

In order to control for varying hygiene levels, initial hygiene was evaluated from the pre-treatment intraoral photographs. The anterior teeth in each arch were assessed by the primary investigator and each arch was graded according to the following criteria (Fig 5):

Good: No plaque or gingival erythema or edema visible on the photograph

Fair: Plaque OR gingival erythema or edema visible on the photograph

Poor: Plaque AND gingival inflammation visible on the photograph

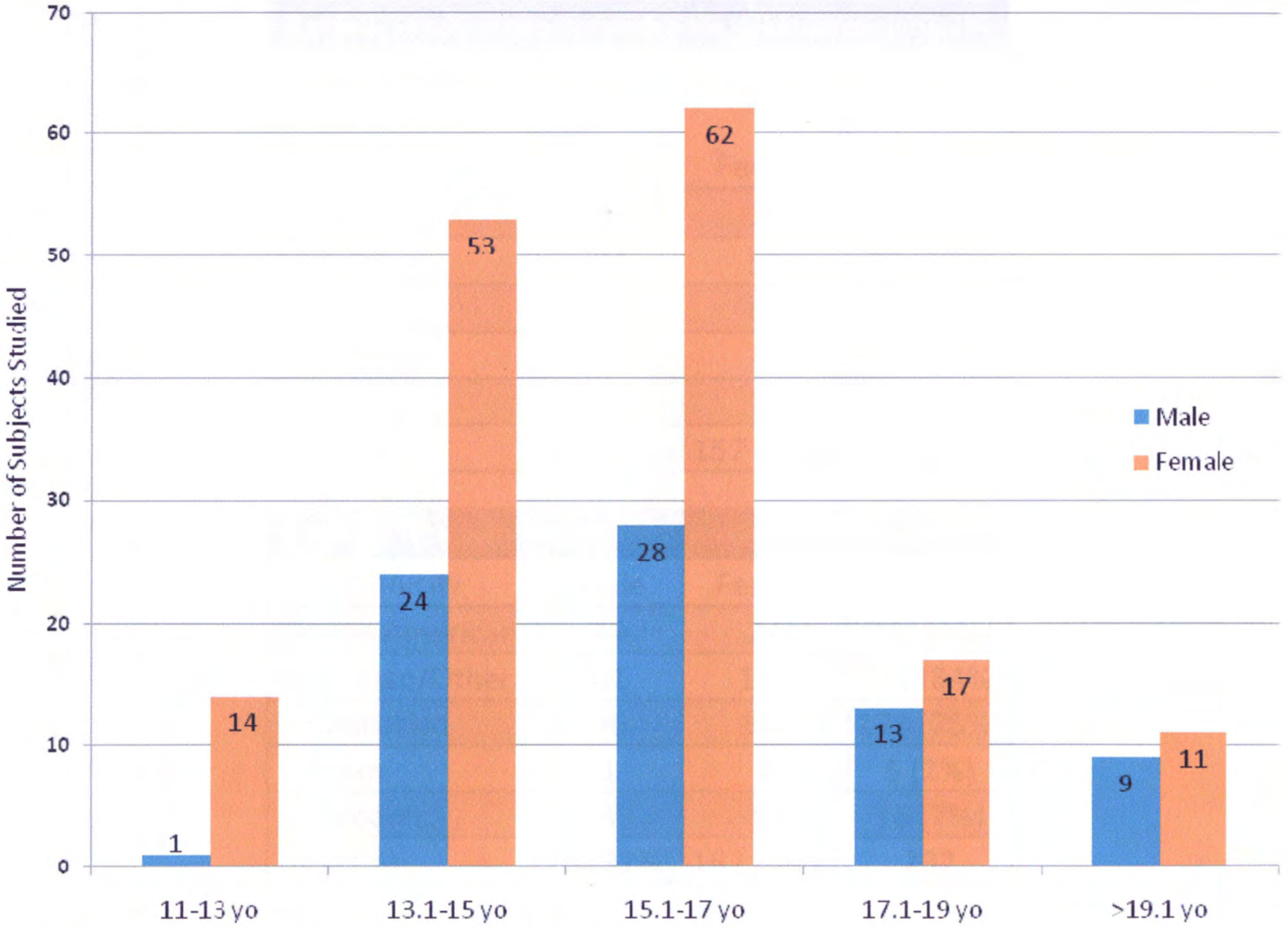


Fig 3. Number of subjects studied by age and gender.

Table I. Age and gender breakdown of subjects studied

Age when completed Orthodontics	Male	Female	Total (%)
11-13 yo	1	14	15 (6%)
13.1-15 yo	24	53	77 (33%)
15.1-17 yo	28	62	90 (39%)
17.1-19 yo	13	17	30 (13%)
>19.1 yo	9	11	20 (9%)
Total (%)	75 (32%)	157 (68%)	232

Table II. Ethnicity and gender breakdown of subjects

Ethnicity	Male	Female	Total (%)
African American	14	24	38 (16%)
Caucasian/Other:	61	133	194 (84%)
Caucasian	56	117	173 (75%)
Asian	1	4	5 (2%)
Hispanic	4	12	16 (7%)
Total (%)	75 (32%)	157 (68%)	232

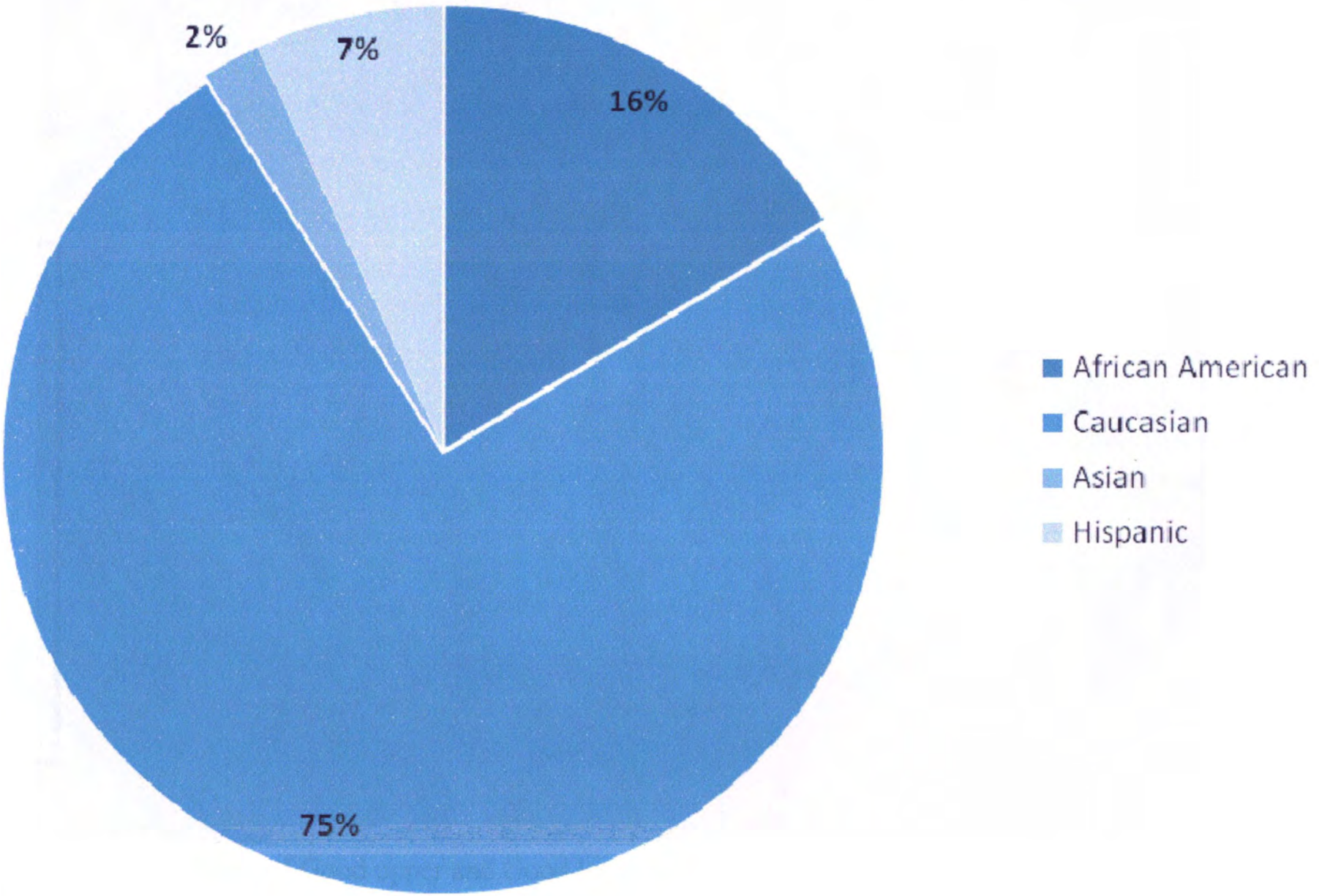


Fig 4. Ethnic breakdown of subjects studied.

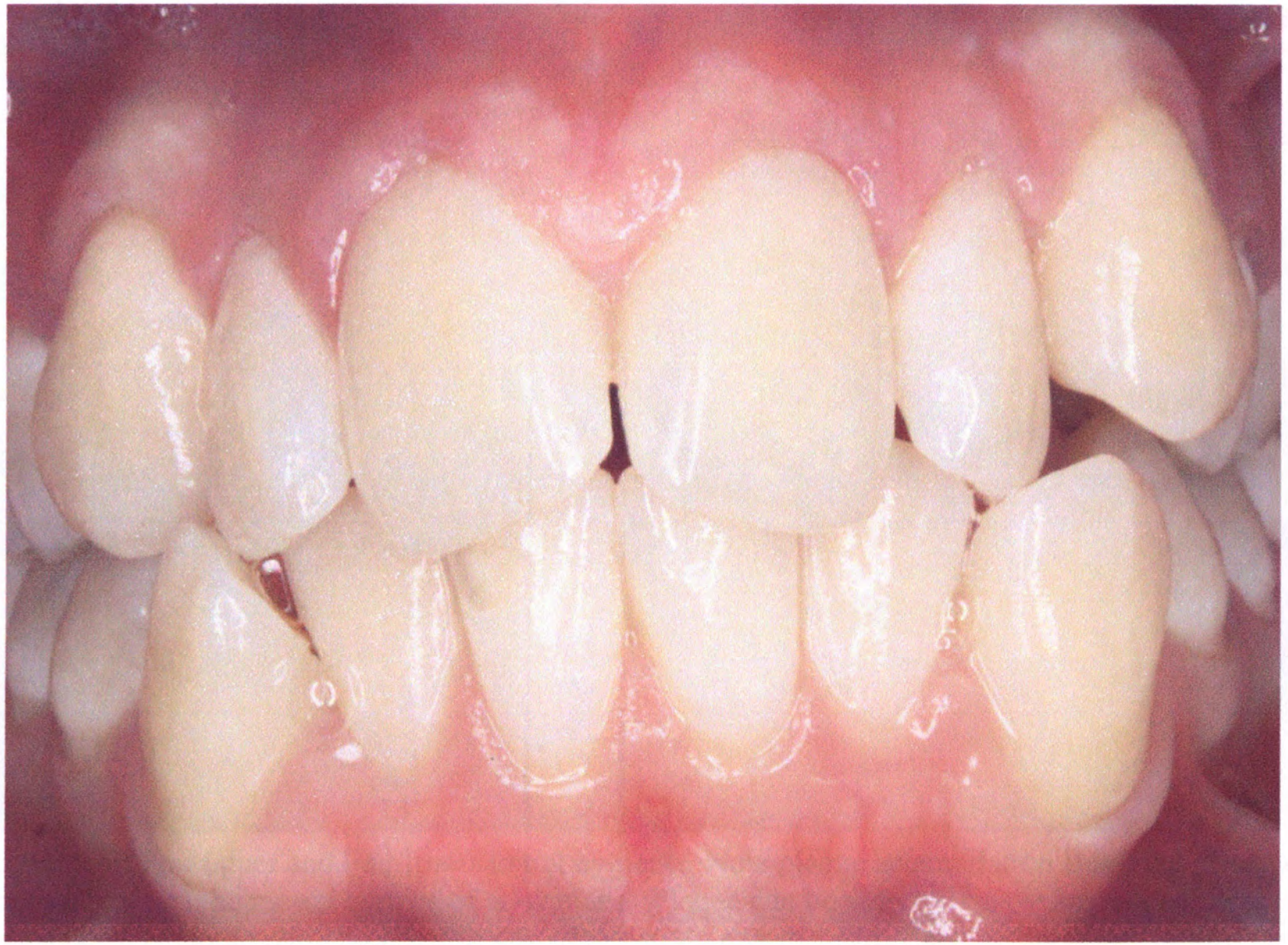


Fig 5A. Example of Good upper and Good lower initial hygiene.

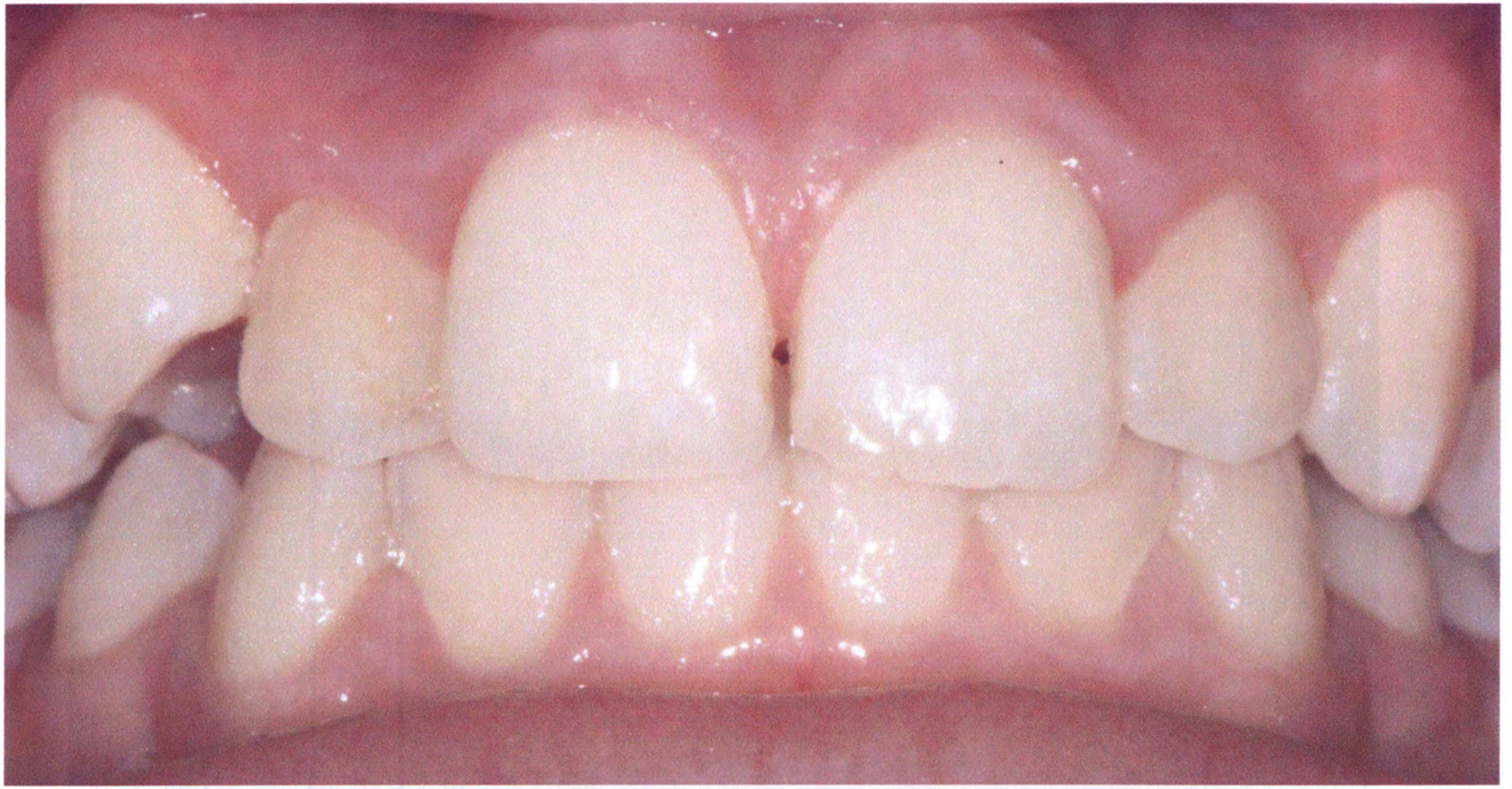


Fig 5B. Example of Fair upper and Fair lower initial hygiene. Note plaque accumulation along the disto-gingival of tooth #7 and on the mesial surface of all canines. Plaque can be detected on the smooth surfaces of several incisors, as well.

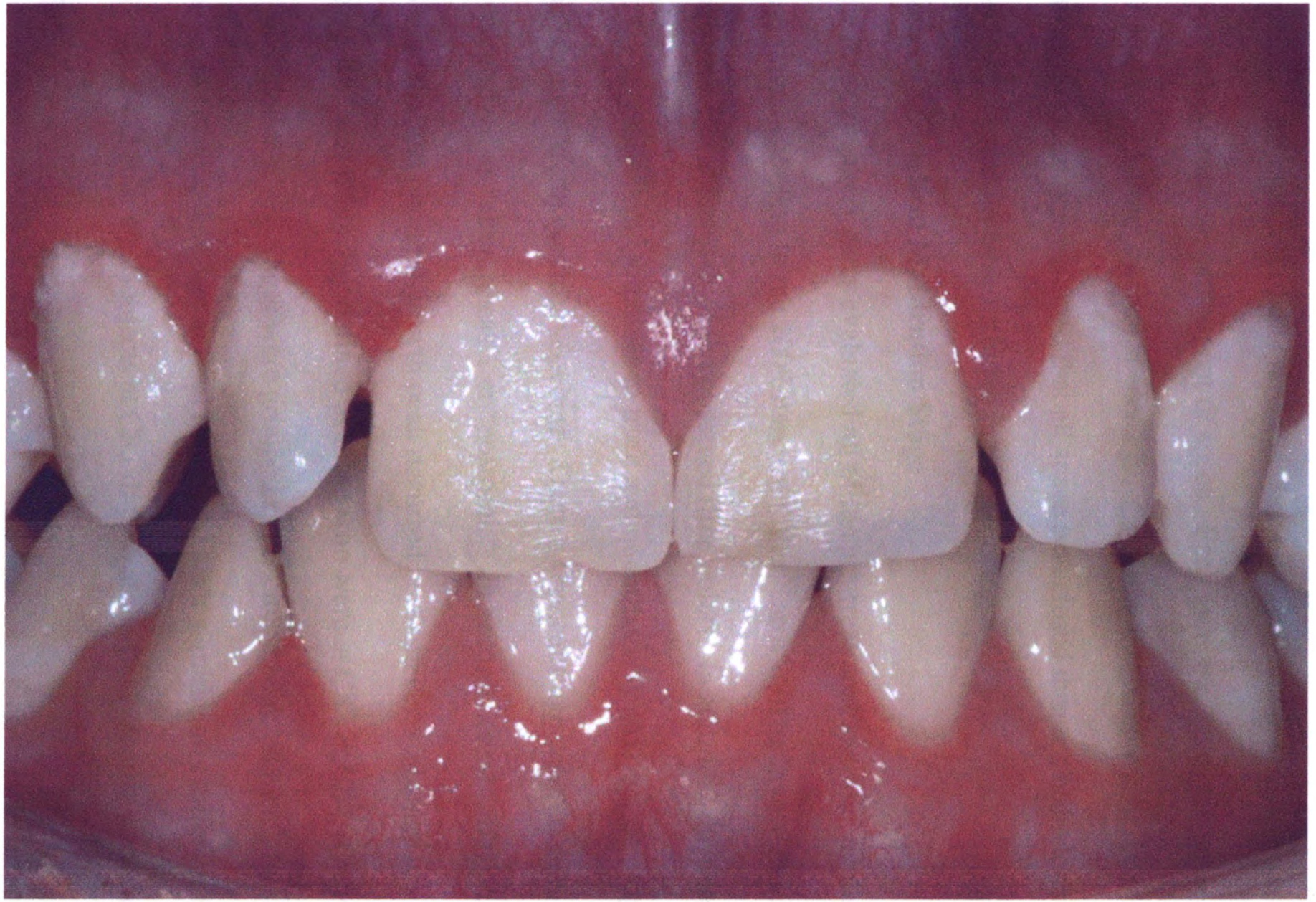


Fig 5C. Example of Poor upper and Poor lower initial hygiene.

Treatment Type: Degree of Space Closure versus Crowding Relief

The amount of space for each site (P1-P10) was recorded from the pre-treatment photographs. All intraoral photographs, occlusal, frontal, and buccal, were used to achieve the best possible assessment of space. Each arch was then categorized as 0 for no space, 1 for 0.5-2mm of total space, 2 for 2.1-5mm of total space, or 3 for greater than 5.1mm of space. It was assumed that all space was closed through orthodontic treatment. Patients with crowding were included in the "0" category.

Cervical Vertebral Maturation Stage

The primary investigator evaluated all of the pre-treatment and final cephalograms for cervical stage according to the 2005 Baccetti CVM[33] article. The investigator had access to the patient age at the each of the observed time points. In addition, the investigator used a CVM analysis in Dolphin® to trace and quantitatively analyze the shape of the second through fourth cervical vertebrae. Once a cervical stage was recorded, subjects were categorized as "circumpeak", or "postpeak". In accordance with Baccetti et al[33] who found that the pubertal growth peak occurred between cervical stage 3 and 4, "circumpeak" was originally defined as those who were at CS3 or CS4. Only six subjects were debonded at CS2. These were grouped with "circumpeak" subjects for ease of analysis and because their age at final records was not significantly different from those at CS3 or CS4 (Table V). Subjects at CS5 or CS6 at the final records were categorized as "postpeak".

Final Gingival Enlargement

For this study, orthodontically-induced gingival enlargement was evaluated at the *final* records for four reasons: (1) cephalograms and intraoral photos are regularly taken together at the final records so the cervical stage could be compared to gingival enlargement, (2) orthodontic treatment has occurred, as opposed to pre-treatment records, (3) a previous image was available to assess and control for hygiene (again, this was not possible if the pre-treatment records had been used), and (4) the appliances have been removed, making quantification of tooth coverage possible. As discussed in the introduction, this investigator was not able to apply existing methods of gingival enlargement directly to a photographic assessment. A modified method for photographically assessing gingival enlargement, termed the Photographic Gingival Enlargement Index (PGEI), was developed and is detailed below.

The ten papilla between the six anterior teeth, both upper and lower, were evaluated (Fig 6). Each site was scored 0 for no gingival enlargement, 1 for moderate gingival enlargement, or 2 for severe gingival enlargement. Due to the variability in lighting conditions when the photographs were taken and adjustments that may have been made when storing the photos in the Dolphin® imaging system, the gingival color was not incorporated into the rating system. Severity of gingival enlargement was graded more on amount of tooth coverage and gingival shape and size. Figure 7 details the PGE Index. Each papillary site was graded individually and then averaged for each arch. All photographs were viewed digitally within the Dolphin® imaging system on a Dell® monitor under constant lighting conditions.

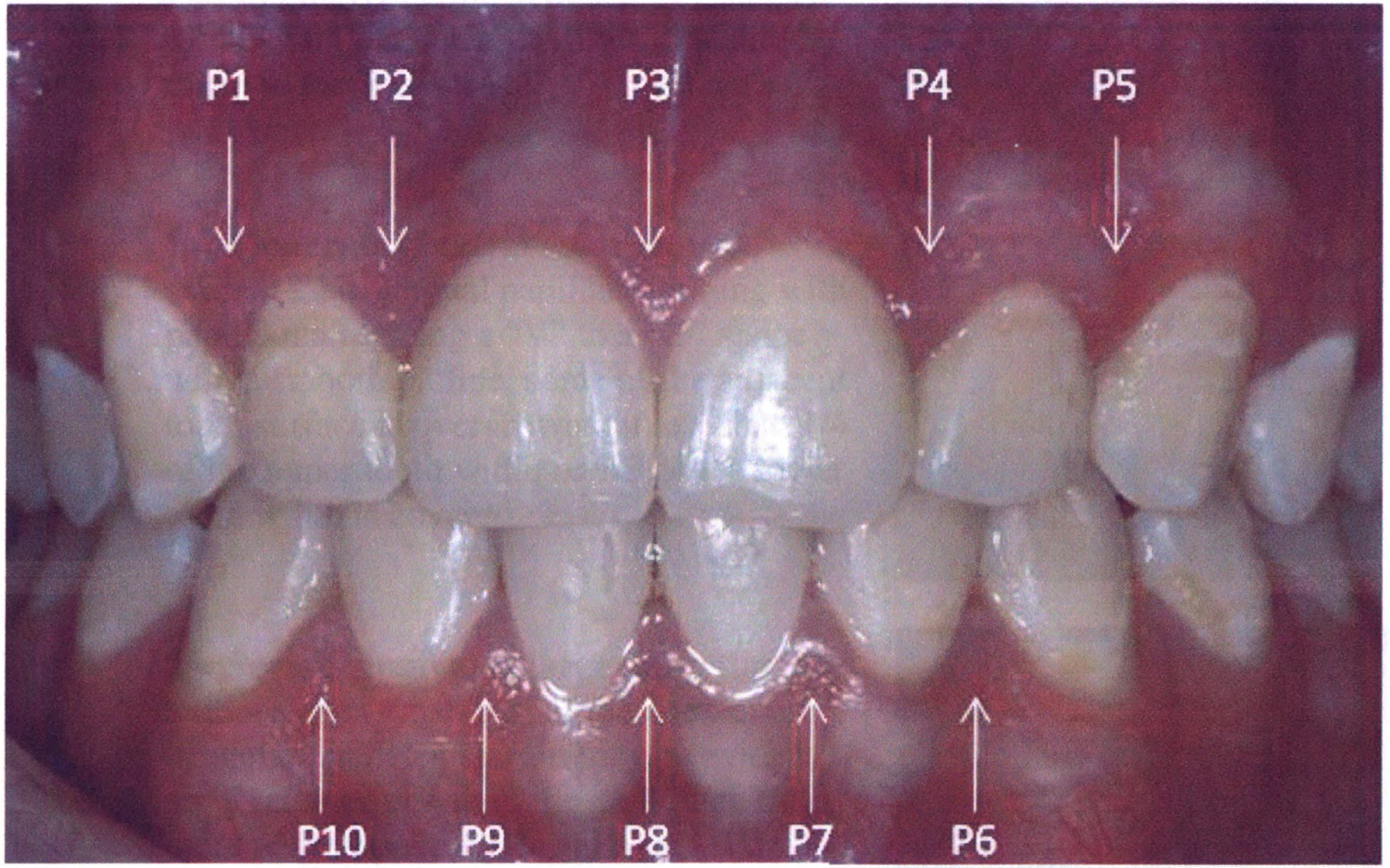
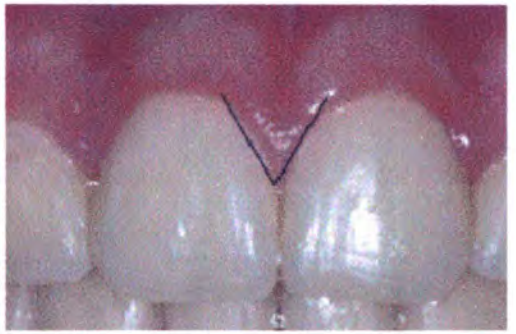
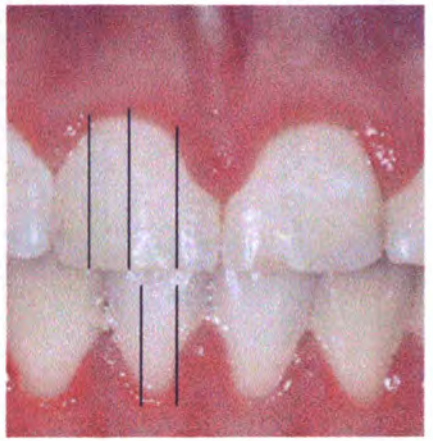


Fig 6. Sites evaluated for gingival enlargement.

0 = No gingival enlargement, "V" shaped papilla with a pointed or slightly rounded apex, no tooth coverage.



1 = Moderate gingival enlargement, evident labio-lingual papillary bulging with papilla maintaining a "V" to a "U" shape with a smooth outline, some of the adjacent tooth surface is overgrown but less than 1/4 of the upper tooth width or 1/3 of the lower tooth width is covered.



2 = Severe gingival enlargement, flat to bulbous apex, "balling" of the apex or an irregular papillary margin may be evident, gingival overgrowth to the point that 1/4 or more of the upper tooth width or 1/3 or more of the lower tooth width is covered.

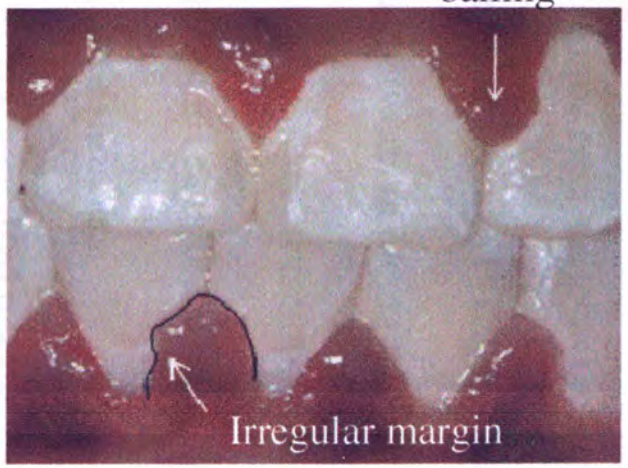


Fig 7. Photographic gingival enlargement index (PGEI)

Statistical Analysis

The primary dataset considered for analysis was the data in which a summary measure of gingival enlargement by arch was collected for each subject (i.e. a subject has two measures of gingival enlargement, such as upper arch average PGEI of 1.5 and lower arch average of PGEI of 0.89). Initially, associations between demographic variables (race, gender, age, cervical stage, treatment length) and oral health factors (initial hygiene, PGEI) were examined using chi-square tests for all categorical variables and t-tests for associations between categorical and continuous variables.

Linear mixed models with a random subject effect were used to examine differences in gingival enlargement by gender, ethnicity, initial oral hygiene, initial spacing, pubertal stage at time of debonding, arch (upper and lower), and length of treatment. Initially, a simple mixed effects regression model was considered and then a multivariable mixed effects model was developed. In the multivariable model, any predictors with $p > 0.20$ were included and a backwards selection was used to determine the final model. All statistical analyses were performed in SAS v 9.2 (SAS Institute, Cary NC) and significance levels was set at $\alpha = 0.05$.

Subsequently, the data in which an ordinal measure (0, 1, or 2) of gingival enlargement is provided for five sites in both upper and lower arches (i.e. a subject has 10 site-specific readings of gingival enlargement) was considered. For site by site analysis, a cumulative logistic mixed regression model was used assuming a multinomial distribution and a cumulative logit link to examine differences in gingival overgrowth by gender, ethnicity, initial oral hygiene, initial spacing, pubertal stage at time of debonding,

arch, and length of treatment. All models included a random subject effect to adjust for multiple measures on each subject. The site-by-site analysis confirmed the initial summary analysis, whose results are detailed below.

RESULTS

Intraexaminer Error

Prior to evaluating all 232 records, the investigator staged the initial and final cephalograms of 50 randomly sampled subjects. Twenty-four hours later, the same 50 subjects were re-staged. Statistical analysis revealed an intraexaminer kappa score 0.906 $p < 0.001$. With kappa scores, agreement is generally considered poor for kappas less than 0.20, fair for kappas, 0.20 to 0.40, moderate for kappa scores 0.40 to 0.60, good for kappas 0.60 to 0.80, and very good agreement if the kappa score falls between 0.80 to 1. Again, prior to evaluating all 232 records, the investigator graded the pre-treatment hygiene of 50 randomly sampled subjects. Twenty four hours later, the same 50 subjects were re-graded. Statistical analysis revealed an intraexaminer kappa score 0.84 ($p < 0.001$), confirming very good agreement between the two time points. As with the other measurements, the intra-examiner error for PGEI by site and by arch was evaluated for 50 subjects, and was found to be very good with the new measurements. The lowest kappa value of 0.755 ($p < 0.001$), which is still considered good, was found for the average score of the lower jaw. When comparing the PGEI at the two evaluation time points for each site, all kappa scores were larger than 0.8 ($p > 0.001$), which is considered very good agreement.

Hygiene Levels

For the upper arch, the initial hygiene was fairly evenly distributed between Good (38.8%), Fair (32.1%), and Poor (29.0%). However, for the lower arch, more subjects were assessed to have Fair (41.3%) initial hygiene than the other categories (Table III; Fig 8). Females tended to have better initial oral hygiene relative to males in both upper and lower arches, $p = 0.002$ and $p = 0.038$ respectively (Table IV). African Americans had significantly better initial oral hygiene in both arches relative to Caucasian/other ethnicities, $p < 0.001$ both arches, good vs. fair/poor (Table IV).

Initial Spacing

There was significantly more initial spacing in the upper arch relative to the lower arch, $p < 0.001$(Fig 9).

Pubertal Stage

Most subjects (69.8%) were debonded at CS5 or CS6, "postpeak." (Figs 10, 11). As shown in Table VI, individuals whose pubertal stage is defined as circumpeak, CS 2-4, are significantly younger than those whose pubertal stage is defined as post-peak, CS 5-6, (14.3 yrs and 16.8 yrs respectively, $p < 0.001$).

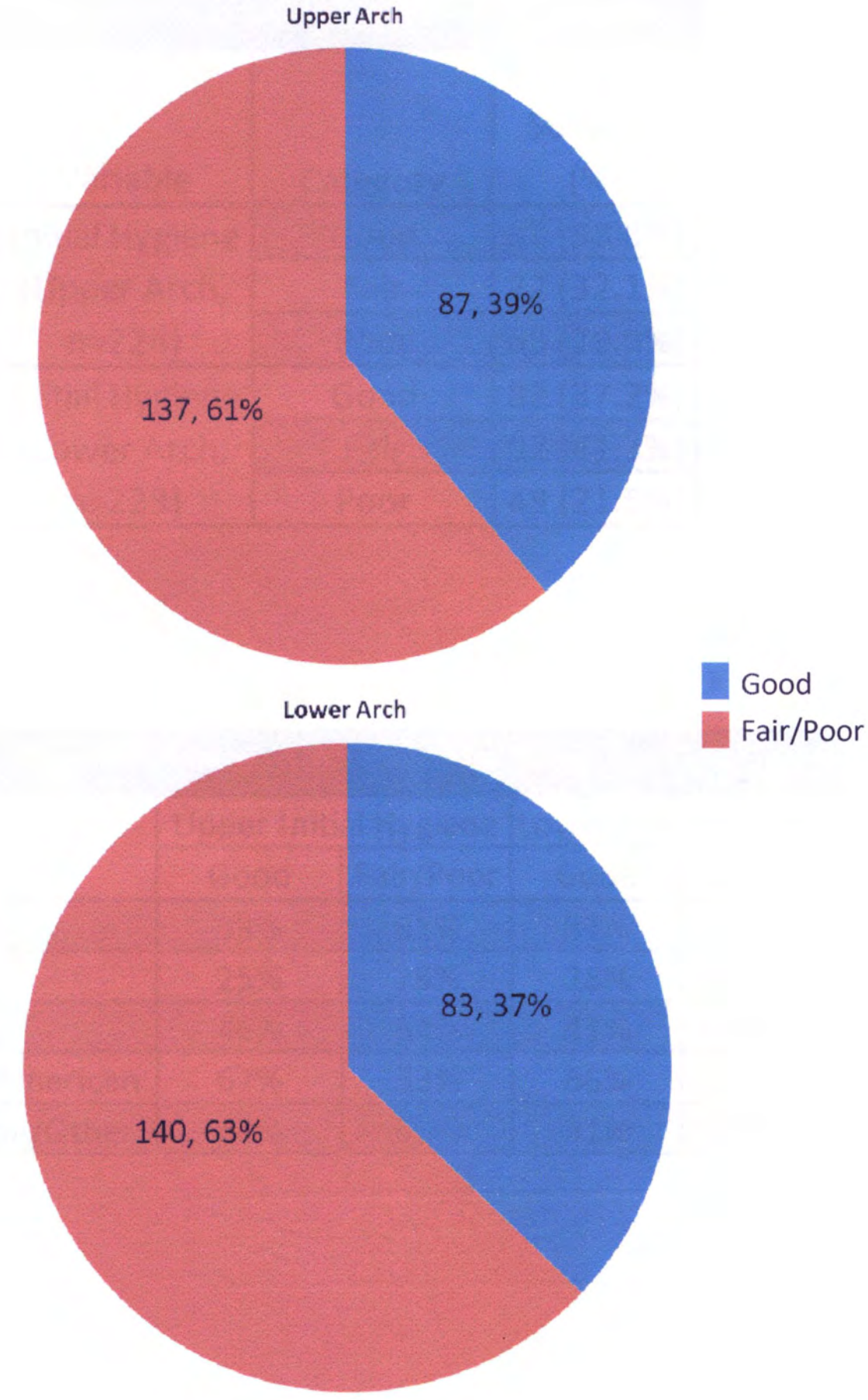


Fig 8. Upper and Lower Arch Hygiene Distribution

Table III. Upper and Lower Arch Hygiene Levels

Variable	Category	Number of Subjects (%)
Initial Hygiene (Upper Arch, n=224)	Good	87 (38.8%)
	Fair	72 (32.1%)
	Poor	65 (29.0%)
Initial Hygiene (Lower Arch, n=223)	Good	83 (37.2%)
	Fair	92 (41.3%)
	Poor	48 (21.5%)

Table IV. Hygiene Levels by Arch, Gender, and Ethnicity

	Upper Initial Hygiene		Lower Initial Hygiene	
	Good	Fair/Poor	Good	Fair/Poor
All	39%	61%	37%	63%
Male	25%	75%	28%	72%
Female	46%	54%	41%	59%
African American	67%	33%	66%	34%
Caucasian/Other	34%	66%	31%	69%

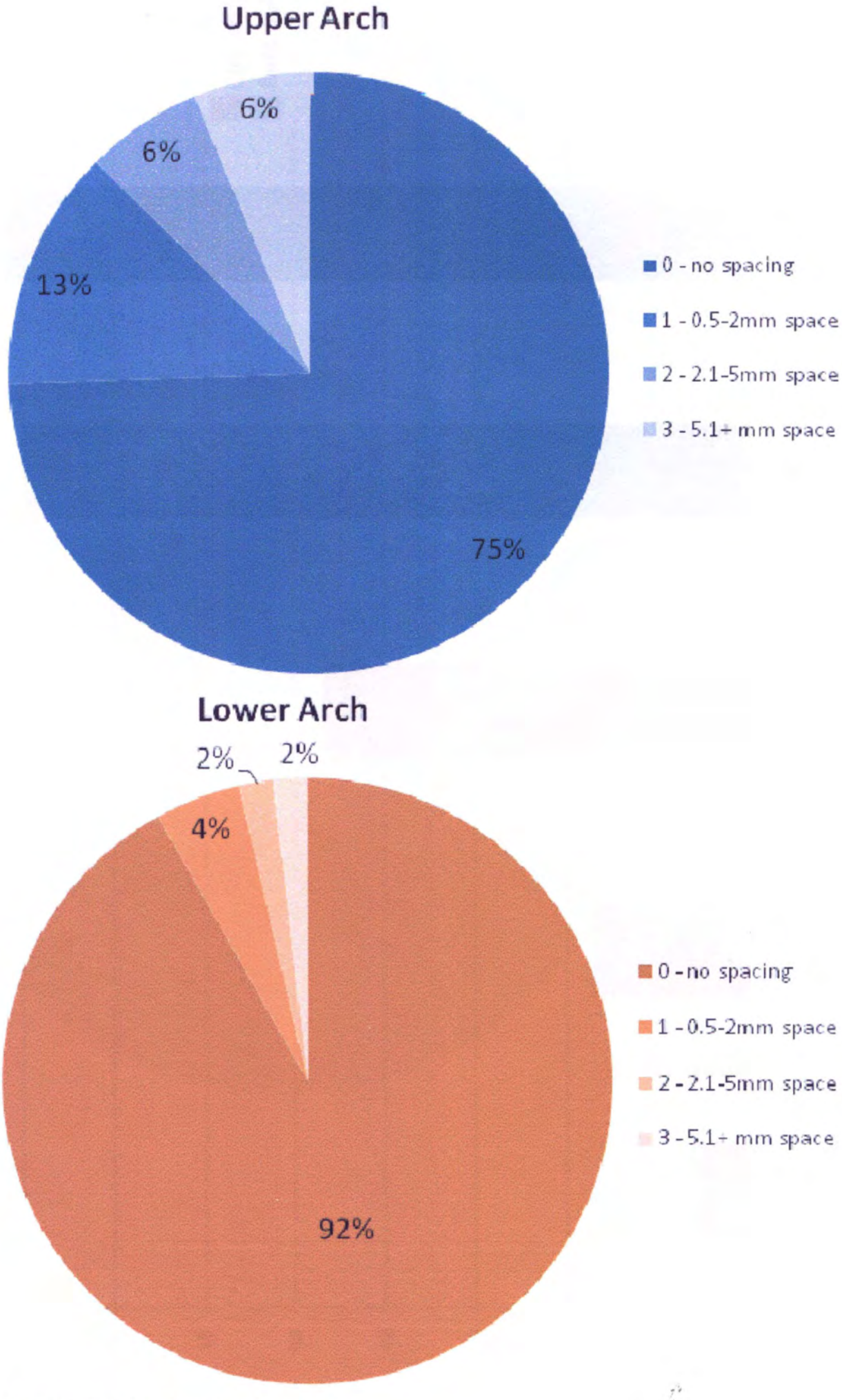


Fig 9. Initial Spacing by Arch

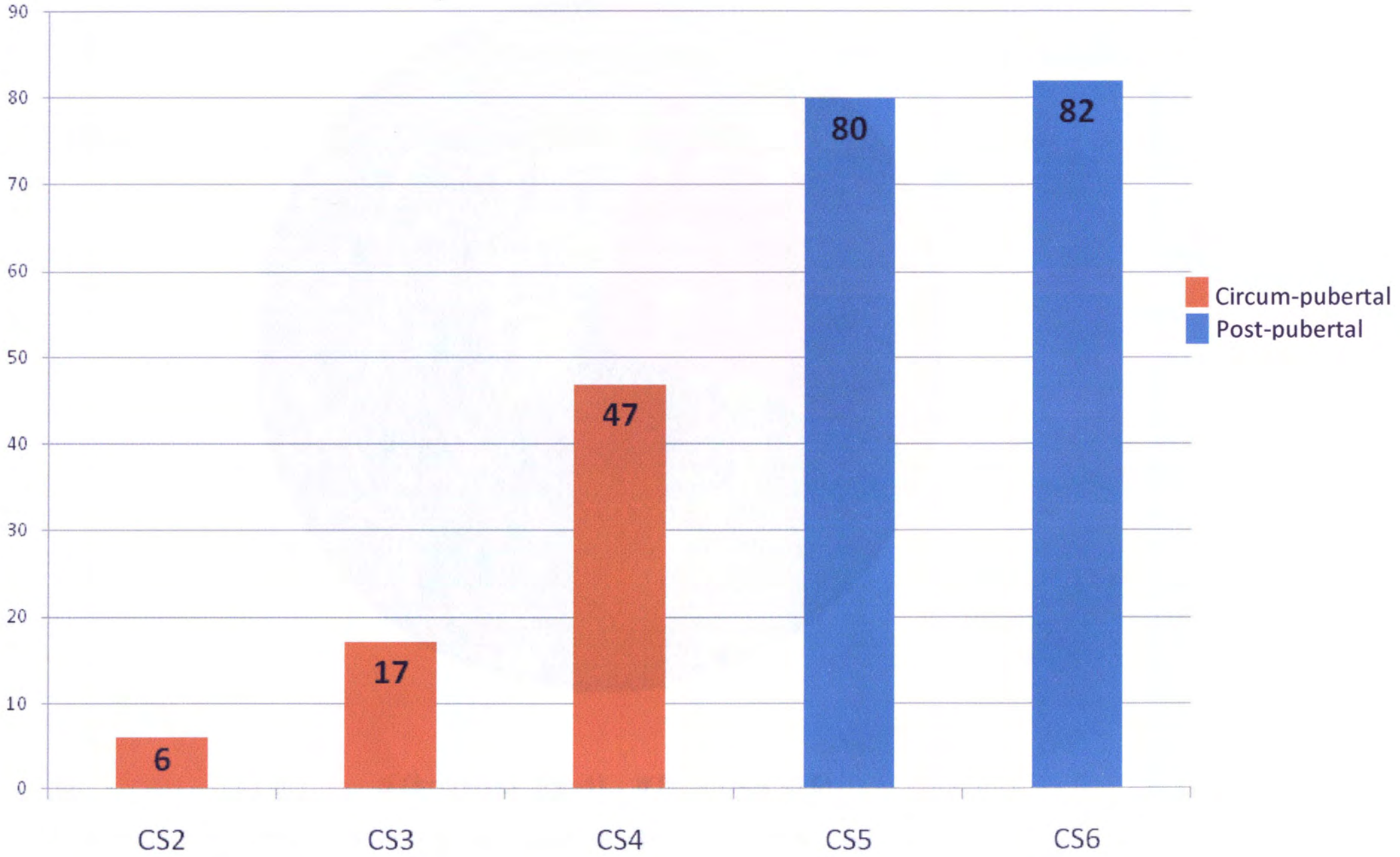


Fig 10. Number of Subjects by Cervical Stage 41

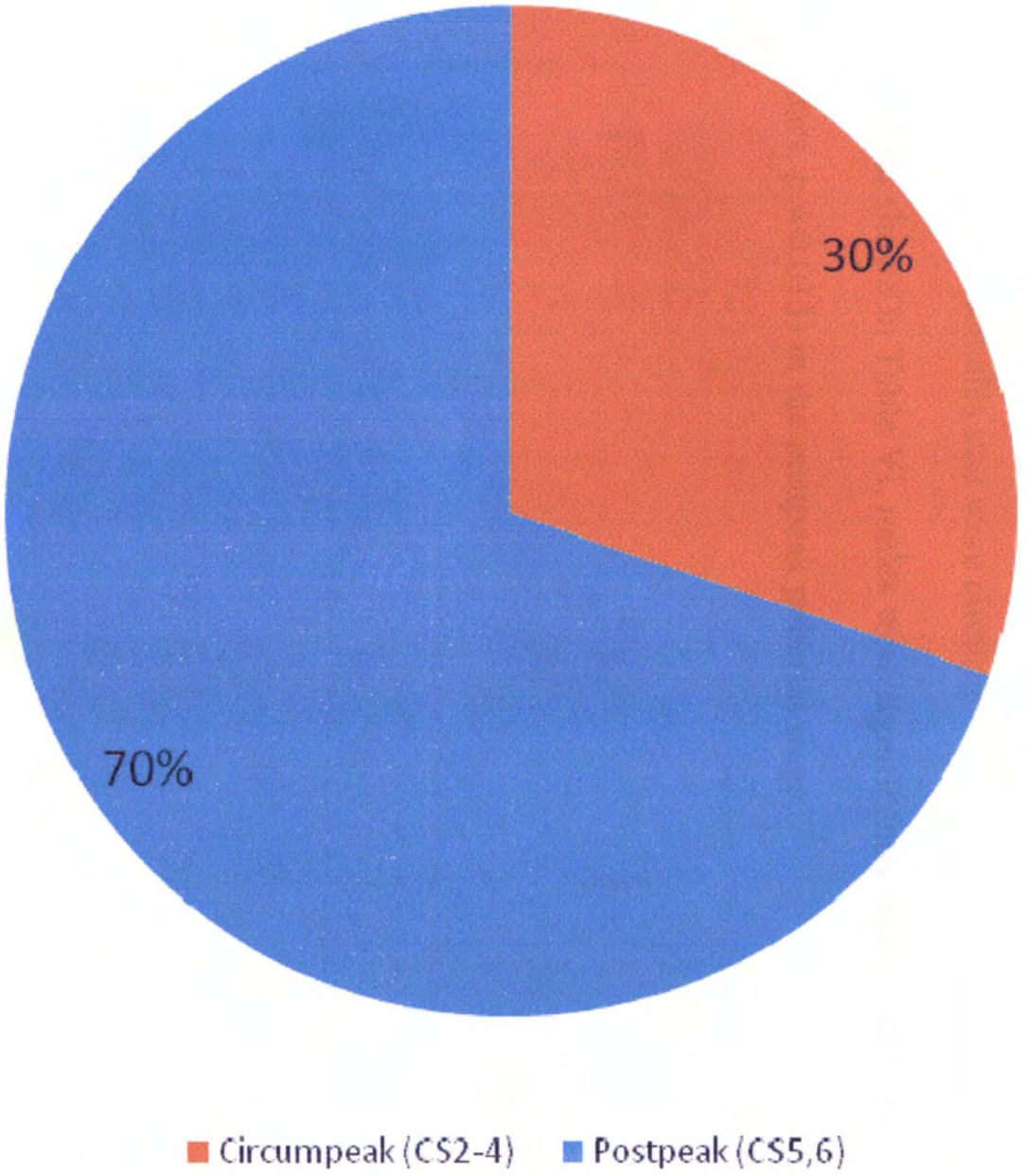


Fig 11. Percentage of subjects debonded at circumpeak and postpeak pubertal stages

Likewise, for African Americans specifically, there was a significant difference in age between circumpeak and postpeak individuals (Table VI). Among individuals in the circumpeak pubertal stage, there was no significant difference in age between African Americans and others (14.4 vs. 14.2 years, $p = 0.989$). There was also no significant difference in age between African Americans and others among subjects defined as postpeak (17.2 vs. 16.4, $p = 0.208$). There was not a significant difference in mean age between males and females that were circumpeak (14.8 vs. 13.9 years, $p = 0.206$). However, as shown in Table VI, males were significantly older than females (17.2 vs. 16.2 years, $p = 0.015$) in the postpeak pubertal stage.

Table V. Mean age (and age range) for each cervical stage

		Gender		Ethnicity	
CS	All	Male	Females	AA	Cauc/Other
2	13.2 (11.7-14.7)	13.6 (12.7-14.7)	12.7 (11.7-13.5)	11.7 (NA)	13.5 (12.7-14.7)
3	14.4 (12.8-16.0)	15.2 (14.4-16.0)	13.7 (12.8-14.7)	15.2 (14.7-16.0)	14.2 (12.8-15.8)
4	14.4 (12.2-16.5)	14.8 (13.7-16.4)	14.2 (12.2-16.5)	14.7 (12.0-16.0)	14.4 (12.4-16.5)
5	15.4 (12.4-18.3)	16.0 (14.3-18.3)	15.0 (12.4-18.2)	15.2 (12.6-17.9)	15.4 (12.4-18.3)
6	17.6 (14.3-24.7)	18.7 (14.6-24.7)	17.3 (14.3-24.5)	19.3 (14.9-24.7)	17.3 (14.3-22.8)

Table VI. Mean age for pubertal category by gender and ethnicity

Category		Circumpeak (n=70)	Postpeak (n=162)	P (ciucum v post)	P CIRCUMPEAK (group1 v group2)	P POSTPEAK (group1 v group2)
All		14.3	16.8	<0.001		
Ethnicity	AA	14.4	17.2	<0.001	0.989	0.208
	Cauc/Oth	14.2	16.4	<0.001		
Gender	Male	14.8	17.2	<0.001	0.206	0.015
	Female	13.9	16.2	<0.001		

Gingival Enlargement

In the primary analysis, composite/average scores of gingival enlargement by arch and the categorical values for initial spacing by arch (0, 1, or 2) were studied. A histogram of the average PGEI scores for all arches reveals that the values are not normally distributed (Fig 13). Most arches had an average PGEI of 0.0, indicating no gingival enlargement at the final records.

Initially, univariate associations were studied between the average gingival enlargement score per arch and gender, ethnicity, pubertal stage, initial oral hygiene, arch, initial spacing within arch, and length of treatment. All variables were considered with a univariate p-value < 0.20 in a linear mixed effects regression model. There was not a significant association between gingival enlargement and gender, ethnicity, pubertal stage at bond removal, or duration of treatment.

The final multivariable mixed regression model of gingival enlargement included significant effects for arch, initial spacing within arch, and initial hygiene. Ethnicity and gender were controlled for in the final model, although neither effect was significant. Arch, initial hygiene, and initial spacing were significant. The mean difference in average gingival enlargement between lower and upper arches was 0.080 units, meaning that the lower arch exhibited a 0.080 increase in the odds of having significantly more gingival enlargement than the lower arch after controlling for other covariates in the model, $p = 0.015$ (Fig 14).

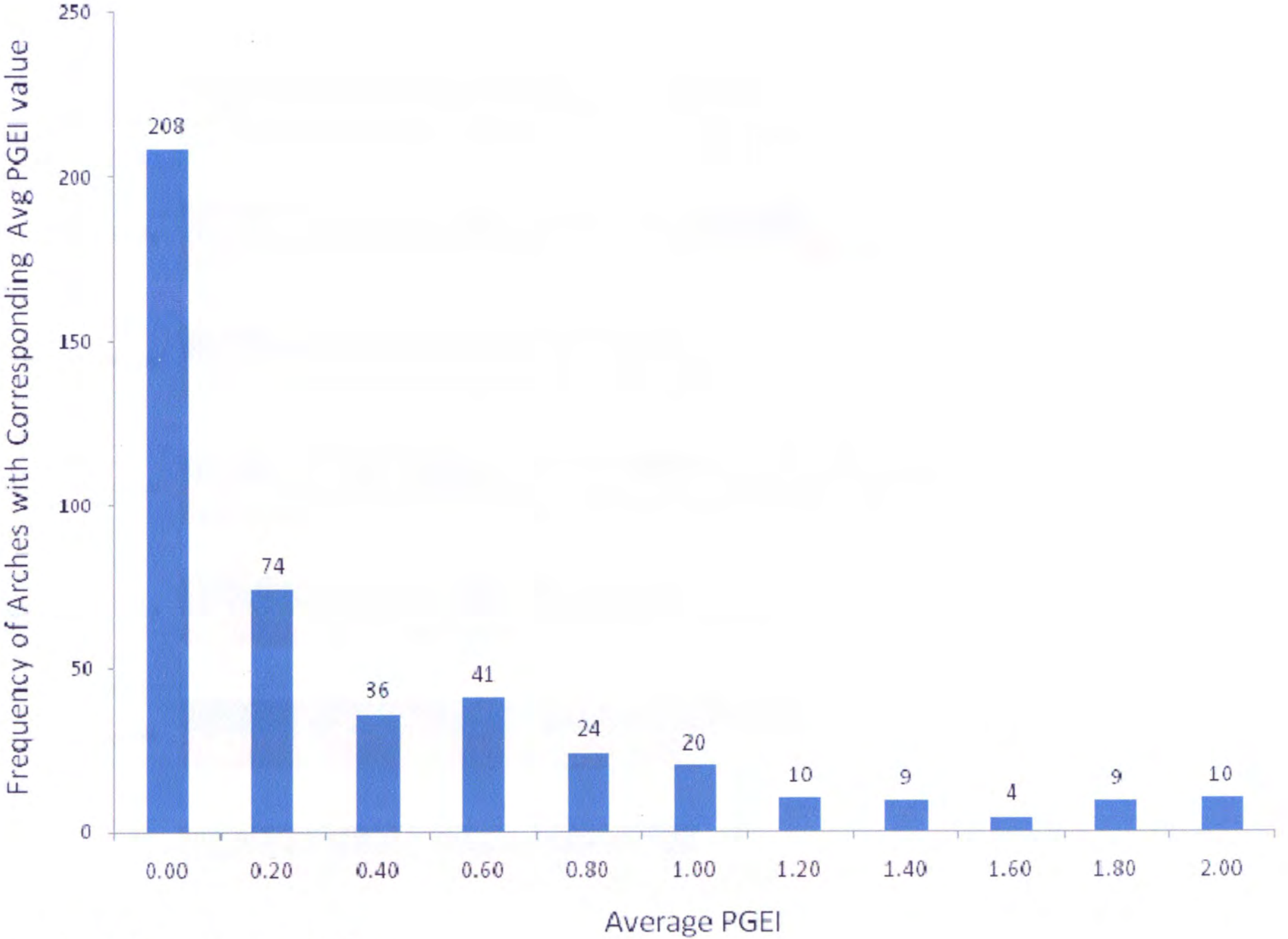


Fig 12. Histogram of PGEI

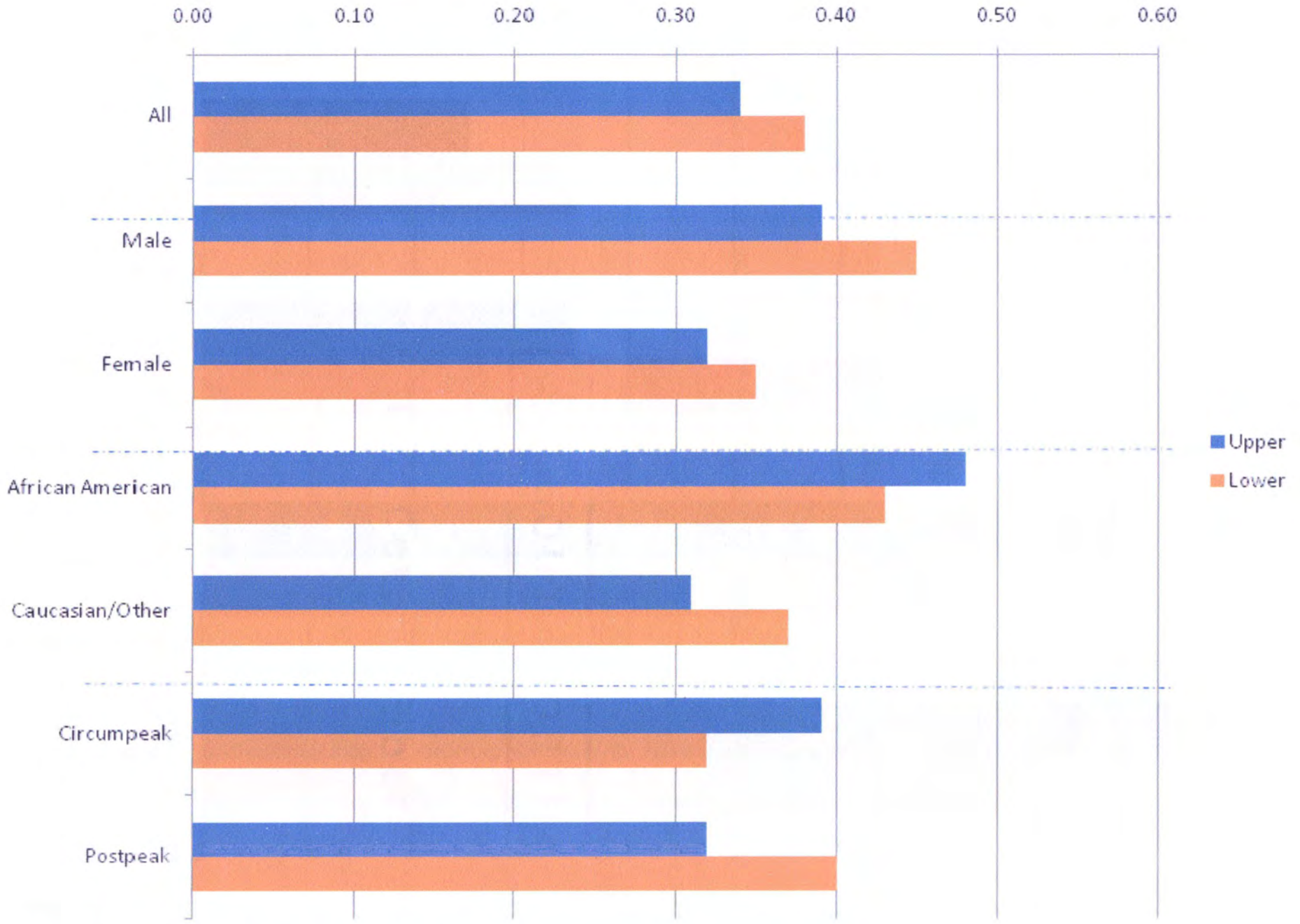


Fig 13. Average PGEI by Arch by Subject Type

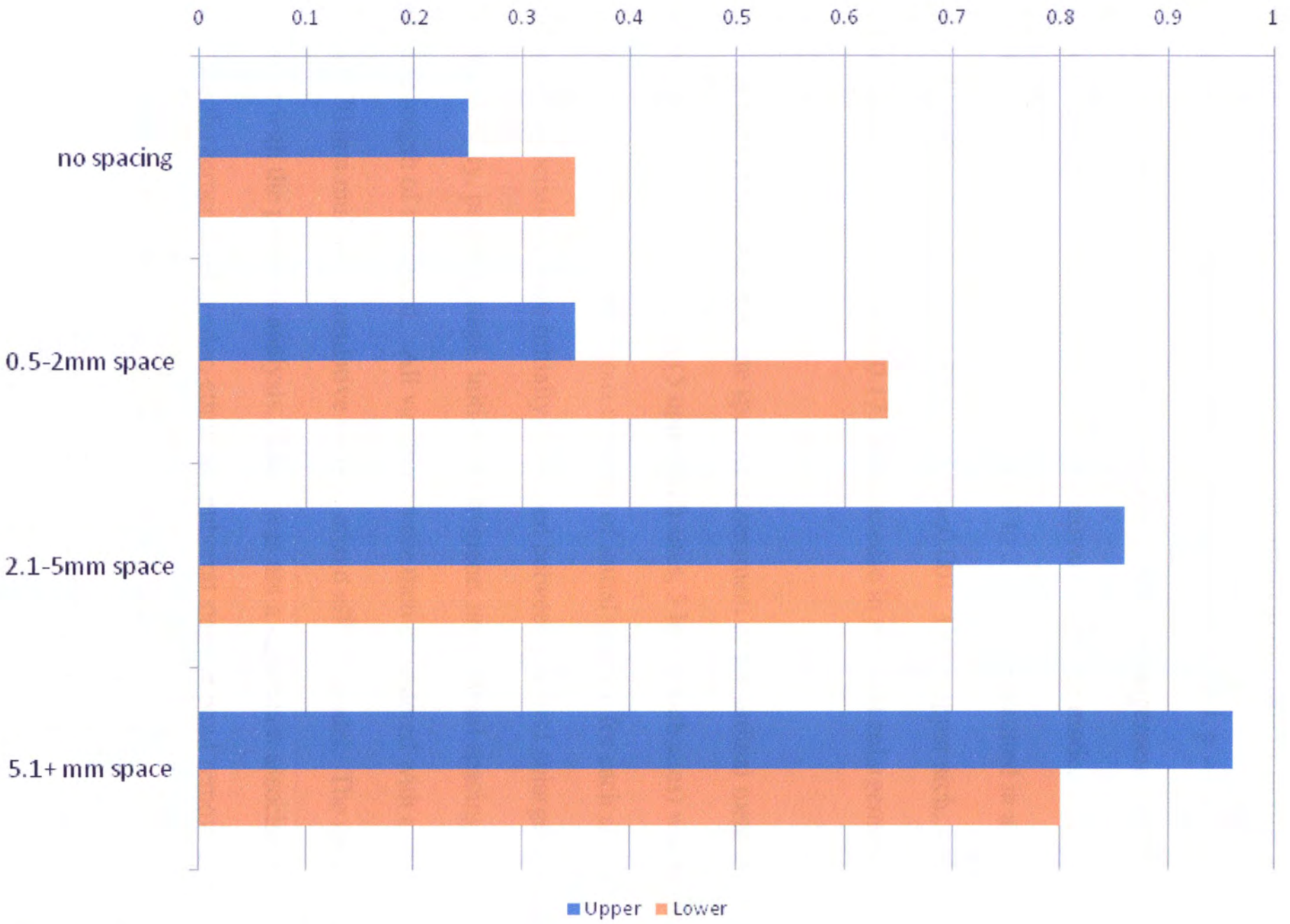


Fig 14. Average PGEI by Spacing Category

The mean difference in gingival enlargement between subjects with good initial hygiene relative to subjects with fair to poor initial hygiene was 0.133 units, meaning that subjects with worse initial hygiene exhibited a 0.133 increase in the odds of having more gingival enlargement when controlling for other covariates in the model, $p = 0.020$. Subjects that started with more initial spacing also had more gingival enlargement relative to subjects with less initial spacing controlling for other covariates in the mode. In the lower arch, an increase from spacing category 0 to 1, or 1 to 2, or 2 to 3 resulted in a 0.215 increase in the odds of having gingival enlargement, $p<0.001$. In the upper arch, a single category increase in spacing resulted in a 0.171 unit increase in gingival enlargement, $p<0.001$ (Fig 15).

In the second analysis, site specific assessment of the ordinal measure of gingival enlargement for each of 10 sites (5 upper arch sites, 5 lower arch sites) was performed. The data also included a continuous measure of initial spacing for each site within arch. Univariate associations were initially examined between gingival enlargement and gender, ethnicity, pubertal stage, initial oral hygiene, arch, initial spacing, site within arch, and length of treatment. All variables were then considered with a univariate p-value <0.20 in a multiple cumulative logistic mixed effects model. The results were consistent with the previous analysis. There was not a significant association between gingival enlargement and gender, ethnicity, pubertal stage at bond removal, or treatment duration. The final cumulative logistic mixed effects model of gingival enlargement included significant effects for initial spacing, initial hygiene, arch, and site within arch. Ethnicity and gender were also controlled for in the final model, although neither effect

was significant. The results were consistent with the previous analysis; fair/poor initial hygiene, initial spacing, and the lower arch were all associated with more gingival enlargement.

DISCUSSION

Gingival Enlargement and Pubertal Stage

Contrary to what was hypothesized, no association was found between cervical stage and severity of gingival enlargement. Obviously, a photographic assessment of gingival enlargement cannot be as accurate as a clinical assessment, with a sampling and cellular analysis of crevicular fluid being the gold standard. Although the PGEI utilized in this research had high intra-examiner agreement, accuracy and reproducibility are yet to be established. In addition, using cervical stage to approximate pubertal hormonal levels introduces several potentially significant sources of error. Accuracy error exists when judging cervical stage[39,40], and variance exists in how closely cervical stage reflects the peak in mandibular growth.[33] The peak in mandibular growth reflects the peak in skeletal growth, but again, variances in individuals exist.[42] Finally, the peak in skeletal growth may not be perfectly correlated with the peak in systemic pubertal hormones. A lag likely exists between the peak in serum sex steroid hormone levels and the observable and measurable growth in skeletal bones. The time required for sex steroid hormones to affect an observable difference in periodontal tissue is yet to be established, as well. With such compounding sources of error, it might be implausible to find a correlation between cervical stage and gingival enlargement. A study that evaluated GI, PI, and gingival crevicular fluid and compared this to either serum or periodontal cellular levels of the sex steroid hormones would eliminate much of this error. Finally, we did not

exclude subjects who were taking medication or who were diagnosed with other systemic conditions known to cause gingival enlargement (idiopathic gingival fibromatosis, leukemic gingival enlargement, plasma cell gingivitis, Wegener's granulomatosis, vitamin C deficiency). It could be argued that this influenced our ability to detect gingival enlargement correlated with increased levels of pubertal hormones, but the percentage of subjects in this age range to meet those descriptions is unlikely to be significant.

Although a correlation between cervical stage and gingival enlargement could not be detected, the age related findings relevant to pubertal category are interesting. Some literature suggests that African Americans tend to reach puberty at earlier ages than Caucasians.[43,44] In our study, African Americans exhibited an *older* average age for both circumpeak and postpeak cervical stages, but these differences were not significant. As expected, males consistently had an older average age for each cervical stage. The difference between males and females was only significant in the postpeak category implying that the males in our study were "circumpeak" at about the same age as females. However, their skeletal growth changes occurred over a longer window, putting them at an older "postpeak" stage. This too is reflected in commonly published pubertal growth charts.[42] Our sample included significantly fewer African Americans (38) than Caucaians/others (194) and significantly fewer males (75) than females (157), so formulating clinical expectations based on these findings is not advisable. Baccetti et al[33] did not offer age ranges for each cervical stage but did tabulate their findings in the 2005 paper. Their sample was based off of 30 subjects, presumably Caucasian, from the

University of Michigan Elementary and Secondary Growth Study conducted from the mid-1930s through the late 1960s. Much research effort is currently focused on recent observations that individuals may be reaching puberty at significantly earlier ages than in previous decades[45-47], including the decades used to establish the CVM index. Although chronologic age is not 100% predictive of growth stage, research establishing current age ranges for the cervical stages might assist orthodontic practitioners in accurately identifying the cervical stage and would provide a baseline for possible future shifts.

Hygiene and it's correlation to Gingival Enlargement

Due to the inflammatory nature of gingival enlargement, finding a correlation between quality of initial hygiene and severity of gingival enlargement is not surprising. Initial hygiene may not be perfectly predictive of hygiene habits during orthodontic treatment, but collecting the hygiene history of each subject was not within the scope of this project. Chapman et al[48] found that initial hygiene is a good predictor of the severity of white spot lesions. In their study, orthodontic treatment did not commence until adequate hygiene was demonstrated. Chapman noted that "even though patients improved their hygiene to start treatment, their previous history of inadequate hygiene placed them at greater risk for white spot lesions, probably because they had a tendency to revert to their bad habits during treatment."[48] Hygiene habits will most likely always be correlated to severity of gingival enlargement, but better controlling for hygiene habits *during* orthodontic treatment might improve the ability to correlate gingival enlargement with pubertal stage in future studies.

In addition to its correlation to gingival enlargement, a few significant differences are worth noting when evaluating initial oral hygiene in relation to subject demographics.

The finding that females had better oral hygiene than male subjects is consistent with most previously published research.[20,21,49] The finding that African Americans had significantly better oral hygiene at the pre-treatment records is somewhat new. Most minority-related research has identified a higher caries incidence and reduced access to dental care for minorities, including African Americans, but has not specifically reported on hygiene differences amongst various ethnicities.[50-52] Most hygiene-related research to date has categorized subjects on socioeconomic status, occupation, and whether subjects come from a disadvantaged neighborhood, as opposed to just ethnicity.[20,49,53] This research has concluded that tooth brushing frequency increases as socioeconomic status improves.[20,54,55] Ethnicity and socioeconomic status are not perfectly correlated, so it may not be appropriate to draw conclusions on specific minority populations based on socioeconomic research findings. In addition, the population of patients at an orthodontic clinic may not be reflective of the demographics and socioeconomics of the population as a whole. In summary, hygiene habits among various ethnicities were not the primary focus of this research, and, although the findings on African American hygiene levels are interesting, more thorough and controlled studies on orthodontic patients are needed before definitive conclusions can be made.

Initial Spacing and It's Correlation to Gingival Enlargement

As with initial hygiene levels, finding a correlation between initial spacing and severity of gingival enlargement was expected. Gingival inflammation is consistently reported in closure of extraction spaces[41,56,57], and there is no reason to anticipate otherwise in closure of genetic spacing.

What was not initially anticipated, however, was that significantly more initial spacing was documented in the upper arch than the lower arch. This could be explained by two possible mechanisms. First, permanent canine eruption was not required in the pre-treatment photographs, but their presence was required in the final records. In the average eruption sequence, maxillary canines are the last tooth to erupt (excluding second and third molars) and can close up to 2mm of upper anterior spacing.[42] Therefore, subjects whose upper permanent canines had not yet erupted at the pre-treatment records might have more maxillary anterior spacing than would otherwise complement the opposing arch. Secondly, studies on the development of the permanent dentition document more crowding in the lower arch than the upper. Moorrees and Chada[58] found that in the general population with normal growth and development, 0.5mm of lower anterior crowding and 0.2mm of anterior space can be expected in the permanent dentition. Gianelly, when reviewing orthodontic models, found significantly more lower anterior crowding, closer to 4.5mm.[59] A natural corollary to these studies would be that, when spacing is present, the upper arch exhibits more anterior spacing than the lower arch in the permanent dentition.

The Lower Arch and It's Correlation to Gingival Enlargement

Explanations for the increased levels of gingival enlargement observed in the lower arch compared to the upper arch are primarily speculative. The gingival enlargement was exacerbated in the lower arch even when controlling for hygiene; the upper and lower arches had comparable percentages of subjects with good as opposed to fair or poor initial hygiene (38.9% and 37.4% respectively). As mentioned above, initial hygiene is not 100% indicative of hygiene during orthodontic treatment. Perhaps lower

arch hygiene during orthodontics worsens more than upper arch hygiene. This concept is not irrational considering the closer bracket proximity to the gingival margin in the lower arch.

SUMMARY AND CONCLUSIONS

Severity of anterior gingival enlargement observed after orthodontic treatment is correlated with the degree of pre-treatment anterior spacing and hygiene, and can be expected to be more severe in the lower arch. Gender, ethnicity, length of treatment, and cervical stage were not found to be correlated with the severity of post-orthodontic anterior gingival enlargement in this study. The photographic gingival enlargement index designed for this study proved to have a high level of repeatability and may be considered for future studies. To better control for the influence of hygiene and pubertal hormone levels, future research should be designed to account for hygiene habits during treatment, to evaluate the gingival condition clinically and at a cellular level, and to evaluate hormone levels through serum or periodontal tissue samples.

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