Age Estimation Through Radiographic Evaluation of Third Molar Mineralization

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THESIS

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This thesis is dedicated to my family Ossama Helal, Pakinam El-Attal, and Shahana Helal, to whom I am grateful for who I am today. The sacrifices they made have allowed me to accomplish my goals and I cannot thank them enough for what they have done.

To the rest of the Helal and El-Attal for their love and prayers. Their love and support are the main reason for the kind of person that I am today and I dedicate all of my accomplishments in life to them.

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LIST OF ABBREVIATIONS

18	Upper Right Third Molar
28	Upper Left Third Molar
38	Lower Left Third Molar
48	Lower Right Third Molar
CI	Confidence Interval
DPSCs	Dental Pulp Stem Cells
IRB	Institutional Review Board
LB	Lower Bound
MSCs	Mesenchymal Stem Cells
Q1	Quadrant 1
Q2	Quadrant 2
Q3	Quadrant 3
Q4	Quadrant 4
SD	Standard Deviation
UIC COD	University of Illinois at Chicago College of Dentistry
UB	Upper Bound

Summary

In forensic examination, observing the stages of the dentition results in highly accurate age assessment but becomes more difficult once dental development is complete. This study evaluated the use of panoramic radiographs to determine chronologic age range utilizing the developmental stages of third molars. A total of 2000 panoramic radiographs of patients between the ages of 4-22 years taken between 2013 and 2015 at the University of Illinois at Chicago College of Dentistry were assessed for inclusion in the study. The development of third molars was classified into eight stages (A-H) according to Demirjian et al.'s classification method. Nine hundred and ninety-nine radiographs were analyzed; differences between the chronological age of third molar mineralization were then compared between sex and quadrants. The subjects' mean age was 13.03 ± 1.68 years for males (392 subjects) and 13.19 ± 1.56 years for females (607 subjects). The majority of subjects did not report ethnicity; however, 213 identified themselves as Hispanics. Hispanic males showed statistically significant lower mean age than Hispanic females at quadrant 4 (Q4) stage B (10.22 ± 1.39 vs 11.67±0.84, p=0.021) and Q1 stage D (12.73 ± 1.19 vs 13.47±1.12, p=0.038). Otherwise, there were no statistically significant mean differences in age between Hispanic males and females for any other classification stage. The 95% confidence interval (CI) on age for each stage in all four quadrants was investigated and a narrow range between upper and lower boundary was observed. This value shows a 95 % probability that the subject true mean age would land within this interval.

The majority of the results indicated no significant mean age differences in third molar mineralization among all four quadrants in both males and females. Panoramic radiographic assessment of third molar mineralization is a useful tool for age estimation in children and young adults.

1. INTRODUCTION

1.1 Background

Forensic age determination is vital to our society. Massive human casualties all around the world have led to unidentified bodies and more methods are needed to help with their identification. More research is needed to further advance our knowledge in this field. Observing dentition stages results in highly accurate age assessment but once dental development is complete, it becomes more difficult to estimate age (Zeng et al., 2010). Hence, with third molars being the last teeth to develop it allows us to further expand our tools in forensic age estimation for young adults. According to the Study Group on Forensic Age diagnostics, in order to improve diagnostic accuracy for age estimation in individuals, several examinations should be conducted. Those include physical examination, dental examination, and hand-wrist radiographs (Schmeling et al., 2008).

Estimation of chronological age through evaluation of third molar mineralization has undergone limited research in the past. Chronological mean age and complete time of third molar mineralization has been inconsistent between both sexes in previous studies. In one study, the mean age of third molar mineralization was earlier in male than that of female (Zeng et al., 2010). On the other hand, a Greek study reported third molars were first detected in females at the age of 7 years whereas they were detected in males at 8 years of

age (Barka et al., 2013). The first sign of detection has been published at different stages by various authors as well, 90% of third molars were visible at the age of 10-11 years (Gorgani et al., 1990), but in a study by Barnett (1976), the first apparent age was 15. A Japanese study reported the first sign of third molar development occurring at the age of 8, while for the Spanish it ranged from ages 5.96-14.66 years. There is no consistent conclusion or estimation on how ethnicity influences the mineralization of third molars or where real differences among different geographic groups might exist; hence a more accurate estimation of chronological age could be provided as a valuable resource.

Third molars have often been impacted due to arch length discrepancies, resulting in clinicians requesting for their extraction (Richardson et al., 1980). There are applications for wisdom teeth that are currently being studied and need to be evaluated further. Dental stem cells can be obtained from developing third molars and have the capacity to differentiate into various types of cells such as, odontoblast –like cells and periodontal ligament cells. By finding the stage of the third molar development where the tooth bud is still viable as the source of epithelial stem cells, narrowing the age range of eligible patients could be achieved (Hadaegh et al., 2014).

1.2 **Objectives**

The objective of this study was to determine the age range of subjects for each developmental stage of third molars in a UIC College of Dentistry patient

population. Specific aims include: determination of differences of the subjects mean age in mineralization stages, sex, race, and quadrant/location.

1.3 Significance

The purpose of this study was to correlate the stages of third molar mineralization (Demirjian et al., 1973) as shown in Figure 1 with the age of the patients in a University of Illinois at Chicago (UIC) College of Dentistry population. Forensic age odontologists can use this as a tool in determining the correct age when applied to individuals involved in massive casualties, those without proper documentation, as well as use in the course of criminal, civil, or asylum proceedings (Schmeling et al., 2008). Furthermore, advances in stem cell research has provided us with more benefit for keeping third molars. Stem cells derived from wisdom teeth can facilitate replacement of other teeth that have been lost due to periodontal disease, caries, trauma, or any congenital disorder. These autologous tissues for dental tissue regeneration can be obtained by harvesting the tooth buds during development and placing them into an embryonic stem cell bank for storage and later use (Zou et al., 2010).

1.4 Hypothesis

There are no significant mean age differences in each stage A-H of third molar mineralization in terms of sex, race, and quadrant/location.

2. REVIEW OF LITERATURE

2.1 Forensic Dentistry

One of the most objective tools in individual identification is through a lifetime of radiographic images of the teeth and jaws giving information of inborn as well as acquired features. It is the leading and most consistent method used for a number of diseases of the tooth/jaw in various ages, in practice of therapeutic and surgical dentistry, and in orthodontic treatment (Bisharyan et al., 2012). Medical and dental documents, dental cast models, photographs, along with dental radiographs are all valuable tools to determine the dental status of an unidentified body. Forensic experts are expected to collect, preserve, and analyze the evidence to present to a legal authority. Dental practitioners around the world have been asked to serve as expert investigators in human identification for criminal purposes. The uniqueness in individual features allows the forensic odontologists to conclude with strong evidence in cases of identification and bite mark analysis, helping to expose the crime perpetrator. Along with other health care providers, forensic odontologists help bring speedy resolutions in cases involving criminal activities such as child abuse, domestic violence, and rape due to more available evidence (Garg et al., 2015). According to Leung et al. (2008), forensic odontology focuses on inspection of three main areas including inspection of: trauma to jaws, teeth, and oral structures, marks to eliminate or identification of a suspect, and dental restorations/prosthesis from anonymous bodies. The firm and resilient form of dental structures like other hard

tissues in the body allows better recognition of what remains even after a severe accident.

Radiographs are extremely valuable in clinical dentistry to diagnose dental disease and formulate treatment plans. In forensic odontology, these records are used mainly in identification and age estimation (Garg et al., 2015). If ante-mortem dental records are not available and other methods are not obtained, the forensic odontologist is essential in identifying information or dental features belonging to the deceased, a process called post-mortem profiling (Leung et al., 2008). There are four main steps in the disaster victim identification process including: finger printing, body tagging, forensic pathology, and forensic dentistry (Garg et al., 2015). Teeth are calcified and are able to withstand fire or other trauma, whereas other physical features are often destroyed, further providing more importance to the forensic odontologist on the team (Avon et al., 2004).

There are cases of asylum-seekers who provided false documentation, complicating the immigration process of registration/identification. In 2004, the countries with the largest number of new refugees were France (58,500), United Kingdom (40,200), Germany (35,600), United States (27,900), South Africa (32,600), Canada (25,800), Austria (24,600), and Sweden (23,100) (Nuzzolese and Vella, 2008). In several countries, age estimation helps in assisting immigration authorities to separate juvenile illegal immigrants from adults (Nuzzolese and Vella, 2008). In some situations, this could help with protecting unaccompanied minors. For example, Italian immigration policies provide residence permits as well as access to education programs if unaccompanied

minors are below the age of 18 (Nuzzolese and Vella, 2008). It is illegal for them to be deported sending them though a juvenile system instead. Many fail to report their actual age, making the process much more difficult for authorities to determine true age. Forensic odontologists contribute to age estimation of asylum seekers relying on clinical and dental examinations along with skeletal maturation as seen on wrist radiographs, root development, and mineralization of third molars seen on panoramic radiographs (Olze et al, 2010).

Research has shown that children of different racial and ethnic backgrounds may develop bones and teeth differently, with systemic conditions causing delay in tooth eruption while on the other hand early extraction of teeth leading to earlier or delayed eruption of teeth. There is a margin of error in all age estimation methods, with no complete data for all ethnic groups. More observational data is needed to assess correction parameters for age estimation formats especially in countries with a high number of asylum seekers (Nuzzolese and Vella, 2008). Assumptions are made regarding the time course of third molar mineralization among various ethnicities, with no clear similarities shown. Thus, it is important to generate statistical results specific for each population due to cross-border migration. Forensic examination is extremely important to be applied to individuals without proper documentation, where age needs to be ascertained during the course of criminal, civil, or asylum proceedings (Olze et al., 2010).

2.2 Chronological age estimation using Demirjian's method of assessing third molar mineralization

Results among various studies regarding dental age estimation through third molar mineralization has varied greatly in the past. Some studies used different methods with some being too subjective. Some methods that have been used in the past include classifications by: Demirjian et. al. (1973), Moorrees et. al. (1963) Gleiser and Hunt (1955), Gustafson and Koch (1974), Harris and Nortje (1983), Kullman et al. (1992), and others.

In 2010 Zeng et al., focused on determining age estimation through radiographic evaluation of third molar mineralization in the Han population of southern China using the Demirjian classification. Upon examining 3,100 Han aged 4.1-26.9 years, their results showed that there was no significant difference between third molars between upper right and upper left or between lower right and lower left of both male and female (Zeng et al., 2010). Significant differences were seen at stage C for females where tooth 28 (upper left third molar) was developing 0.25 years earlier than 38 (lower left third molar). At stage G for males, tooth 38 was 0.61 years earlier than 28 and tooth 48 (lower right third molar) was 0.62 years earlier than 18 (upper right third molar). The mean age and complete time of third molar mineralization of male overall were earlier than female. They compared their study to other ethnicities finding major differences and were unable to give reasons as to why this occurs suggesting that more studies are needed to resolve this issue. Chronological mineralization age of tooth 48 at stages D to G of Han was 1-4.6 years earlier than Japanese and 1-3

years earlier than the German population. At stage H, the age was similar to Turkish, Black African, Japanese, and German, but was later than in Spanish population (Zeng et al., 2010).

Monirifard et al. (2015) radiographically assessed third molar development and their relation to chronological age in an Iranian population. After analyzing 505 panoramic radiographs of which 223 were male and 282 were female aged between 6-17 years, they reported that all third molars showed a highly significant correlation with dental age; with the mandibular left third molar being the highest for males and the mandibular right in females (Monirifard et al., 2015). It was concluded that third molar calcification can be used to estimate age with the mandibular teeth being more reliable for both genders in all ages. This radiographic method can be used for deceased as well as living individuals having significant advantages in not needing to prepare microscopic sections, no need for extractions, and no need for expensive/sophisticated histology or laboratory equipment. When compared to previous studies, results were similar stating no significant differences in relation to the sex of the patient further supporting studies by Araujo et al. (2010), but were in contrast with other studies such as with Legovic et al. in 2010 (Monirifard et al., 2015). This study further proved that calcification stages should be used as an additional tool to estimate chronological age. In 2011, Costacurta et al. found strong correlation between dental and chronological age with molar calcification stages supporting previous results. At stage D, the mean age for individuals was reported at 13.62 years of age, which was similar to the Turkish population at 12.90, but less than the

Japanese population at 18.2 for male and 18.0 for female. It was also less than the German population at 16.3 for male and 15.5 for female, further proving significant differences between ethnicities (Monirifard et. al., 2015).

2.3 **Demirjian's method vs. others**

Dental age is valuable to the orthodontist in treatment planning various malocclusions in relation to maxillofacial growth, but it can also provide help in determining age of cadavers or other skeletal material, as well as diagnosing pediatric endocrinopathies (Demirjian et al., 1973). Demirjian et. al. (1973) wanted to provide a new system for dental age assessment through radiological appearances. As opposed to other methods each stage has been rated according to developmental criteria such as, amount of dentinal deposit, shape change of the pulp chamber, and amount of root formation. To reach such results 1446 boys and 1482 girls of French Canadian heritage were analyzed and third molar mineralization stages were divided into A-H. Several studies investigated the relationship between emergence and root formation, stating that it has varied between different teeth. In studies conducted by Liliequist and Lundberg (1971), they concluded that tooth formation is a more reliable indicator of dental maturity than gingival emergence or eruption. Demirjian developed a system to define stages not by their length measurement, but rather by estimating the overall maturity of the tooth, giving a complete description for each stage. It is a revised version to that of (Tanner et al., 1973), where four trained examiners evaluated panoramic radiographs and a set of scores was expressed for each stage of

each tooth. Demirjian et al. (1973) concluded that it is safe to assume that the pattern of development of the teeth will vary in different populations, whereas the maturity scoring system is a valid measuring instrument for universal use.

In 2005, Olze et al. tested several methods to determine the most accurate results regarding age estimation through evaluating third molar mineralization. The team analyzed 420 panoramic radiographs of German females aged 12-25 years and looked specifically at tooth 38. Methods used included: Gleiser and Hunt (1995), Demirjian et al. (1973), Gustafson and Koch (1974), Harris and Nortje (1984), and Kullman et al. (1992). Gustafson and Koch's method (1974) was the only one that presented a 4-stage written classification system, the others had diagrammatic representation as well as written description. Good results were developed by Gleiser and Hunt as well as Kullman et al., but the highest intra-class and eta coefficient was shown by Demirjian et al. in 1973 (Olze et al., 2005). All authors except for Demirjian et al. used stages based on metric measurements of root or length, whereas Demirjian et al. (1973) had stages defined by changes in form. Results showed that the Liliequist and Lundberg in 1971 method showed low accuracy among all age groups, where Gustafson and Koch in 1974 was highly accurate for male subjects only. It was concluded that the most accurate results were obtained by the Demirjian et al. (1973) classification system, providing the best results for both the observers as well as correlation between true and estimated age (Olze et al., 2005). Demirjian provided sufficient number of stages that was neither too long nor too short. The classification method also did not need to provide

analysis through a metric system, “This leads to the conclusion that the method devised by Demirjian et al. (1973) should be used for evaluating the mineralization of third molars for purposes of forensic age determination” (Olze et al., 2005).

Staaf et al. (1991) examined 541 conventional orthopantomograms of patients aged 5.5 to 15.5 years and tested three methods. Those classifications included: Demirjian et al. (1973), Haavikko (1970), and Liliequist and Lundberg (1971). Age of both genders was overestimated by 6-10 months when using Demirjian et al. classification system, while the other two methods showed a systematic underestimation or overestimation by 6-7 and 7 months, respectively (Staaf et al., 1991; Olze et al., 2005). The authors stated that the method provided by Demirjian et al. could have proved less valid due to ethnic differences between their study and their original study. On the other hand, Mörnstad et al. in 1994 analyzed 197 conventional orthopantomograms by 13 independent observers from Swedish children comparing various methods, showing that the highest accuracy compared with real age was obtained by (Demirjian et al., 1973). In 2005, Olze et al. stated that it is extremely difficult to ignore differences in sample size, age group, age distribution, ethnic origin, or medical history when comparing studies as they could influence some of the results and question their validity. The use of kappa coefficients and eta coefficients has allowed the analysis of the different stages independent of various populations. They approved the use of the Demirjian et al. (1973) method stating, “Of the various methods examined, Demirjian et al.’s classification

achieved the highest values for both observer agreement and for correlation between the stages defined by the method and true age. It can, therefore, be regarded as the best method".

Alshiri et al. (2016) evaluated Western Saudi Arabian children using three methods to determine their accuracy. Those methods included: London Atlas of Tooth Development, Demirjian's dental maturity scale, and Mincer's method. They found that all three methods under estimated age by 19, 14.5, and 26.5 months respectively. The London Atlas predicted 65.1% of the subjects' age within 12 months, in contrast to 28.5% using Mincer's method, and 71.4% using Demirjian's method (Alshiri et al., 2016). They then concluded that Demirjian's method estimated age more precisely than the other two methods, claiming that estimated age was closest to chronological age.

Reproducibility of radiographic stage assessment of the developing third molar was studied by Dhanjal et al. in 2006, where intra- and inter-observer reliability was tested. Intra-observer agreement and percentage agreement was highest for Demirjian et al.'s method for mandibular molars, as it was the only method showing substantial agreement with considerably higher percentage than other methods (Dhanjal et al., 2006). Third molars are unique compared to the rest of the dentition, being more variable in size, shape, sequence of formation, and eruption. Their roots are less divergent and in some cases fused making it more difficult to accurately assess their root length, hence Demirjian's method provided more reproducible results due to analyzing the apical root cone angle instead of its measurement. Reproducibility is better for mandibular than for

maxillary third molars due to superimposition of anatomical structures in the maxilla such as the hard palate, floor of the maxillary antrum, inferior border of the zygomatic arch, soft tissue, and other artifacts (Dhanjal et al., 2006). When compared to other studies such as Moorrees et al., Demirjian's classification method showed the highest kappa value. An increase in the number of stages might have improved accuracy giving the investigator more concise options to choose from but at the same time it could result in less precise data due to an increase in error. Furthermore, it was concluded that Demirjian's method of stage assessment of third molars showed very good agreement for both intra- and inter observer analysis due to clearly defined stages and fewer intermediate stages producing an improvement in reproducibility (Dhanjal et al., 2006).

2.4 Use of third molars as stem cell sources

Dental epithelial stem cells are located at the incisor labial apical end where it is essential for its ability to produce enamel and induce dentin formation (Dassule et al., 1998). According to Dassule et al., germ cells can be obtained from third molar teeth when patient is five years old, and it can be reserved in a liquid nitrogen profound hypothermia container (-196 degrees C) in order to preserve them for many years. The multi-potential characteristics of each germ cell will help determine the ability of a tooth to differentiate into various types of morphology. Additional growth factors, such as FGF, BMP, or PDGF along with scaffold material help to facilitate the differentiation of those stem cells when seeded in the jaw (Dassule et al., 1998).

Stem cells have been found in teeth holding the potential to treat various conditions including: Type I diabetes, neuronal degenerative disorders, cardiovascular diseases, paralysis due to spinal cord injury, liver disease, stroke, and others (Karbanová et al., 2010). Adult stem cells that are able to proliferate and differentiate without ethical or legal concerns as multipotent mesenchymal stromal cells (MSCs) are great resources for tissue bioengineering (Hadaegh et al., 2014). MSCs are isolated from bone marrow, and can also be obtained from adipose tissue, umbilical cord, and dental pulp (Gronthos et al., 2000; Shafiei et al., 2014). Previous studies have shown that size and shape of the tooth crown result from epithelial morphogenesis during the development stages of bud, cap, and bell. During the bud stage, the dental epithelium separates into two histologically distinct cell lineages, the peripheral basal cells contacting the basement membrane, and the stellate reticulum, which are derived from the suprabasal cell layers of the surface ectoderm. These tissue layers combined form the epithelial components of the stem cell niche in the continuously growing teeth (Thesleff et al., 2009).

3. MATERIALS AND METHODS

This study was approved by the University of Illinois at Chicago (UIC) Institutional Review Board, Office of the Protection of Research Subjects (OPRS), IRB Protocol #2015-0892. Development of the third molar mineralization stage for each subject radiograph was recorded by the principal investigator to evaluate the subjects' age by sex, ethnicity and quadrant.

3.1 Study Design

A total of 2,000 conventional panoramic radiographs with subject ages between 4 and 22 years old treated at the University of Illinois at Chicago College of Dentistry (UIC COD) from year 2013 to 2015 were assessed for inclusion in this study.

The development and mineralization of third molars was classified into eight stages (A-H) based on Demirjian's method. The first four stages (A-D) show crown calcification from the appearance of cusp to completion of crown, and second four stages (E-H) show root formations from radicular bifurcation beginning to apical closing (Demirjian et al., 1973). According to published literature with a sample of approximately 500 subjects, the study had a power of at least 80% (type error I of 5%) to detect standard deviation mean difference between the groups (Zeng et al., 2010).

3.2 **Sample Selection: Inclusion and Exclusion Criteria**

Radiographs were examined by a co-investigator to determine their eligibility according to a set of inclusion/exclusion criteria. Eligible radiographs were de-identified by the co-investigator in order to maintain confidentiality and protect patients' identity. The patients' ethnicity, sex, and age in years were recorded by the co-investigator and kept in a separate folder until the data collection was complete. The sample had to meet the inclusion/exclusion criteria below.

Inclusion criteria:

- Subjects between 4-22 years
- Non-distorted panoramic radiographs
- Normal growth, no medical conditions affecting dental development

Exclusion criteria:

- Patients who had third molars extracted
- Patients with significant medical history: i.e. congenital abnormalities, drug usage, etc.
- Images showing dental pathology such as cysts and tumors

The de-identified radiographs were labeled with subject numbers and transmitted to the primary investigator who then recorded the third molar stage of mineralization for each quadrant according to the Demirjian method.

3.3 **Methods**

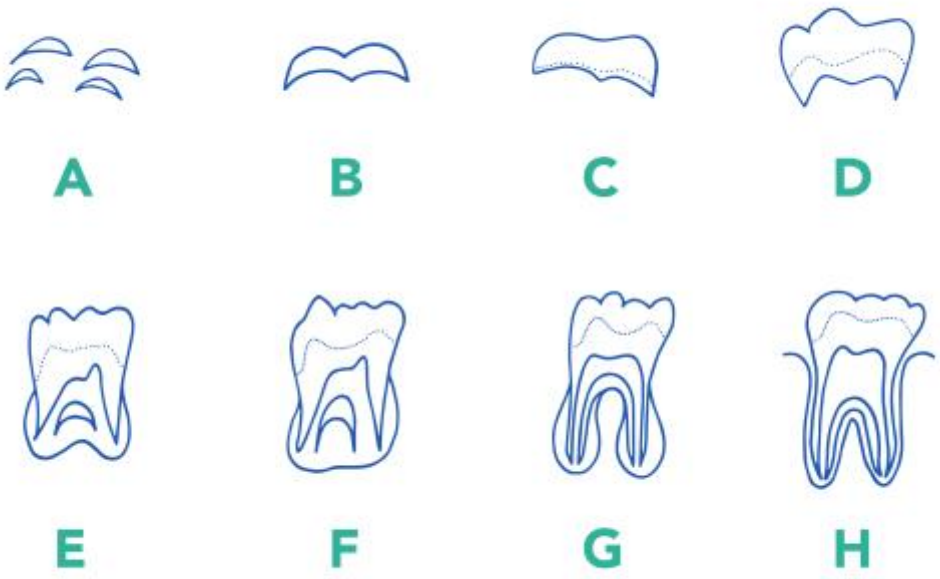
Panoramic radiographs were taken during the course of treatment in the Orthodontic and Pediatric Dentistry Departments at the UIC COD utilizing the Orthopantomograph OP 300 and OP 200 machines, respectively. The radiological images were taken between 2013 and 2015. Two thousand radiographs were narrowed down to 999 after filtering them through inclusion and exclusion criteria and exported through Dexis Digital Radiography. The co-investigator kept all radiographs in a folder electronically after having been de-identified. Age, race, and sex were recorded for each individual patient at the end once the Demirjian developmental stage was recorded for all radiographs. The age of the patient was calculated in years at the time the radiograph was taken. For example: John Smith, African American, Male, 20 years and 4 months old was coded as subject 1- AA M 20.33 and given to the principal investigator de-identified as subject 1.

Once the principal investigator received de-identified coded radiographs, tooth mineralization of all third molars was evaluated according to the method introduced by Demirjian et al. (1973) for each quadrant. All assessments were performed on the same computer with the same contrast/resolution settings. A spreadsheet was used with subject number in rows, each of the four quadrants in columns. Each developmental stage was recorded from one to ten. One was recorded if the third molar was completely absent and two was recorded if a follicle existed with no evidence of tooth mineralization. Three to ten represented A-H respectively (Figure 1). To minimize bias, age, ethnicity, and sex were filled

out in the spreadsheet by principal investigator once all radiographs had been completely evaluated. In terms of sex identification, one was coded as male and two as female. Each race was given a numerical code as: African American-1, Caucasian-2, Asian-3, Hispanic-4 and Other-5. Assessment of 50 radiographs selected randomly by principal investigator were analyzed to provide intra-reliability strength.

3.3.1 **Study Variables**

Stages of third molar mineralization A-H, according to Demirjian et al. 1973.



Stage A: Mineralized cusp tips, but have not merged.
Stage B: Mineralized cusps have merged resulting in a more well-defined coronal morphology.
Stage C: Crown is half-formed; the pulp chamber is evident with initiation of dentinal deposition.
Stage D: Crown formation is complete to the dentinoenamel junction (DEJ).

Stage E: Formation of the inter-radicular bifurcation has started. Root length is less than the crown length.
Stage F: Root length is as great as crown length.
Stage G: Root walls have developed, but apices remain open.
Stage H: Root apices are completely closed.

Figure 1: Mineralization stages A-H of third molars according to Demirjian et al. (1973)

3.4 **Statistical Analysis**

The assumption of normal distribution was verified using the Shapiro-Wilk test. Mean, standard deviation and 95% Confidence Interval (C.I.) of the variable age in years were obtained. Descriptive statistics were reported as frequencies and proportions for categorical variables: race, sex, and developmental stage of third molar. Distribution of the developmental stages among the four quadrants was evaluated using nonparametric Friedman test. Independent Student t-tests were used to compare the mean age differences on each stage of development between sex. All calculations and tests were performed with IBM SPSS Statistics for Windows (version 22.0, IBM Corp., Armonk NY). Statistical significance was set at 0.05.

4. RESULTS

4.1 Study Sample

Two thousand radiographs were available for assessment. Based on the inclusion/exclusion criteria, 999 subjects' radiographs selected for this study, showing ethnicity distribution as: 39 African American, 19 Caucasian, 26 Asian, 213 Hispanic, 2 other, and 700 did not record their ethnicity as shown in Table I. Ethnicities were not reported by student dentist or resident in the majority of subjects, hence the only two categories recorded in this study with sufficient data to analyze were Hispanics and a combination of all ethnicities.

4.2 Reliability Testing

Fifty randomly selected radiographs were evaluated twice one week apart to assess the principal investigator's intra-observer agreement on the third molar mineralization stage in each quadrant. Cross tabulations and Cohen's Kappa coefficients indicated a consistent agreement (around 90%) and a Kappa coefficient of higher than 0.80, indicating a good intra-reliability by the investigator for the method used in this study.

4.3 Descriptive Statistics

Overall, there were 392 (39%) males and 607 (61%) females. Mean age was 13.07 years ranging from 6.50 to 16.75 years. Table II shows mean age of male at 13.03 years with 95% confidence interval ranging from 12.66 years to

13.40 years, where female mean age showed to be at 13.19 with 95% confidence interval ranging from 12.93 years to 13.45 years. Third molars were missing in 123 subjects in quadrant 1, 127 subjects in quadrant 2, 108 in quadrant 3, and 103 in quadrant 4 as shown in Table III.

TABLE I

DEMOGRAPHIC CHARACTERISTICS OF THE STUDY SAMPLE

Race	**Frequency**	**Percent**
African American	39	3.9
Caucasian	19	1.9
Asian	26	2.6
Hispanic	213	21.3
Other	2	0.2
Total	299	29.9
Missing Race	700	70.1

TABLE II

SUBJECT AGE AT THE TIME RADIOGRAPH WAS TAKEN

Gender		**Age**	**Std. Error**
Male	Mean	13.03	0.18559
	95% Confidence Interval Lower Bound	12.66	
	95% Confidence Interval Upper Bound	13.40	
	Std. Deviation	1.68	
Female	Mean	13.19	0.13316
	95% Confidence Interval Lower Bound	12.93	
	95% Confidence Interval Upper Bound	13.45	
	Std. Deviation	1.56	

TABLE III

THIRD MOLAR OCCURENCE FOR EACH QUADRANT

		Quadrant 1	**Quadrant 2**	**Quadrant 3**	**Quadrant 4**
N	**Present**	876	872	891	896
	Missing	123	127	108	103

4.4.1 **Quadrant 1 Analysis**

Of 876 subjects (mean age, 13.20 ± 1.56) years at Q1 (quadrant 1), 175 Hispanic subjects (mean age, 13.05 ± 1.57) years was the only race in the sample enough for statistical comparisons. The mean age of Q1 Hispanic males showed statistically significant lower mean than for females in stage D, (p-value= 0.038). Otherwise, there were no statistically significant mean differences in age (years) between Hispanics male and females in other Q1 stages, (p>0.05). Figure 2 illustrates mean age of subjects at various stages for all races and figure 3 shows same data solely for Hispanics.

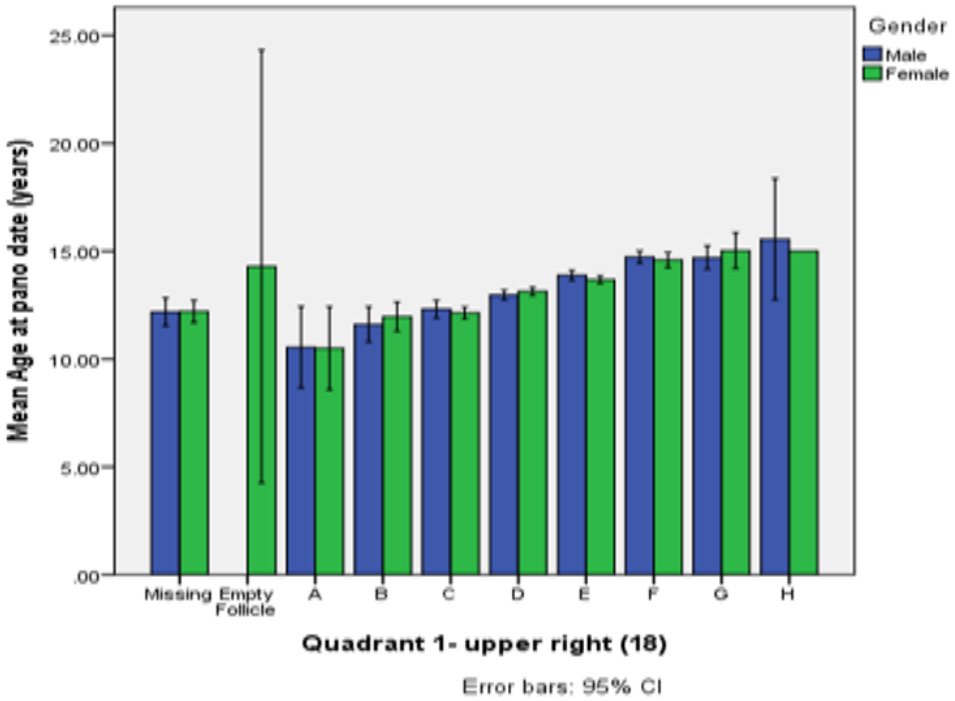


Figure 2: Mean age for all races in quadrant 1

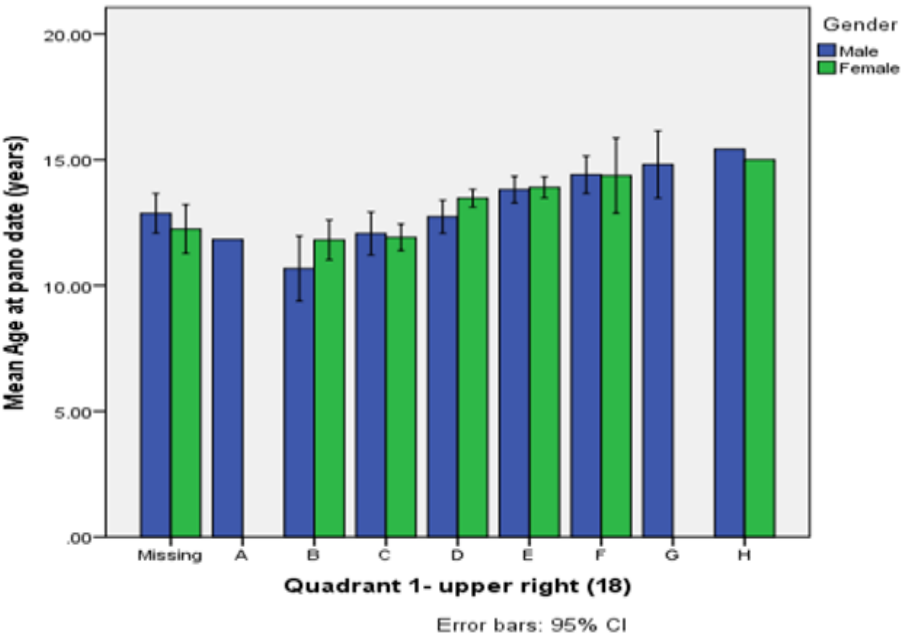


Figure 3: Mean age for Hispanics in quadrant 1

4.4.2 **Quadrant 2 Analysis**

Of 872 total subjects (mean age, 13.20 ± 1.56) years at Q2 (quadrant 2), 178 Hispanic subjects (mean age,13.03 ± 1.54) years was the only race sample enough for statistical comparisons. The mean age sample between males and females did not indicate statistically significant mean differences at any stage, (p-value >0.05). Figures 4 and 5 illustrate mean age of subjects at various stages for all races and for Hispanics only, respectively.

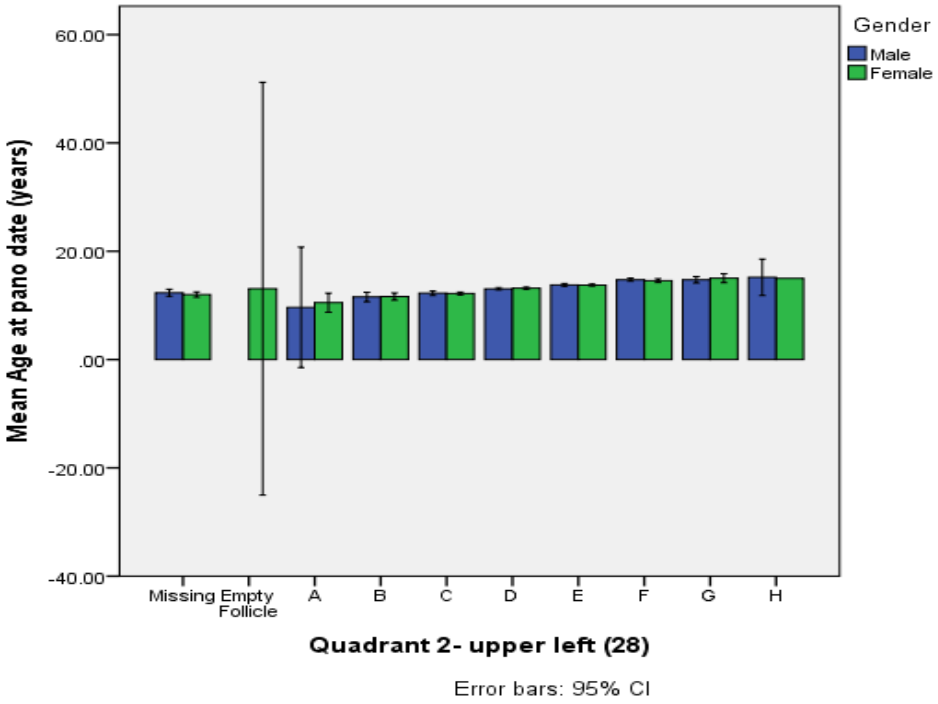


Figure 4: Mean age for all races in quadrant 2

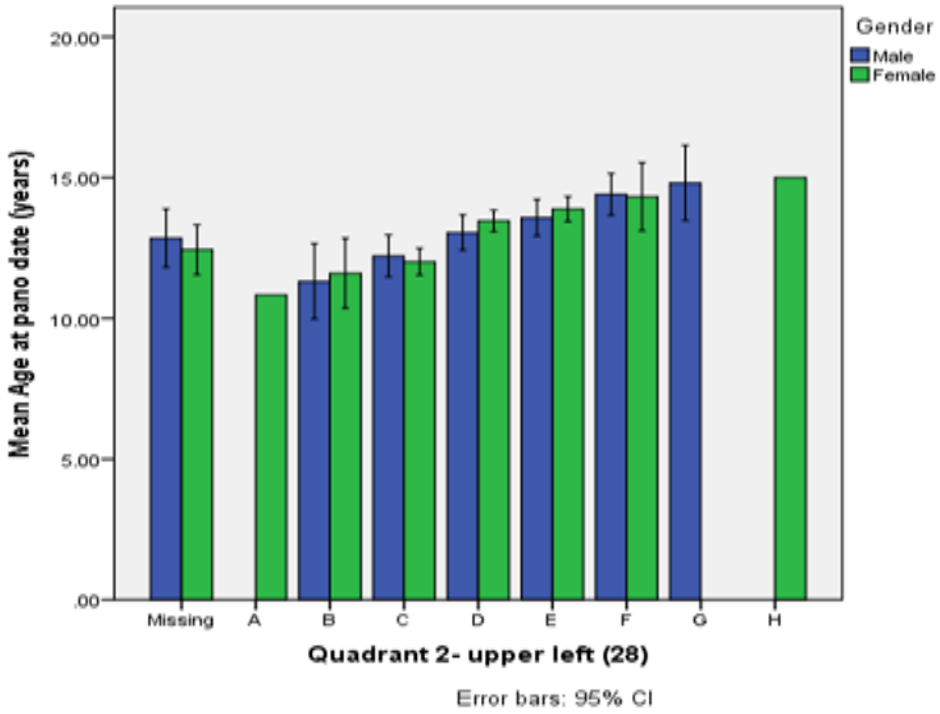


Figure 5: Mean age for Hispanics in quadrant 2

4.4.3 **Quadrant 3 Analysis**

Of 891 total subjects (mean age, 13.13 ± 1.63) years at Q3 (quadrant 3), 190 Hispanic subjects (mean age, 12.96 ± 1.59) years was the only race with enough sample for statistical comparisons. In Q3, the mean age of males showed statistically significant lower mean than for females in stage B, (p-value= 0.009). Otherwise, there were no statistically significant mean differences in age (years) between male and females in Q3 stages, (p>0.05). Figures 6 and 7 illustrate mean age of subjects at various stages for all races and for Hispanics only, respectively.

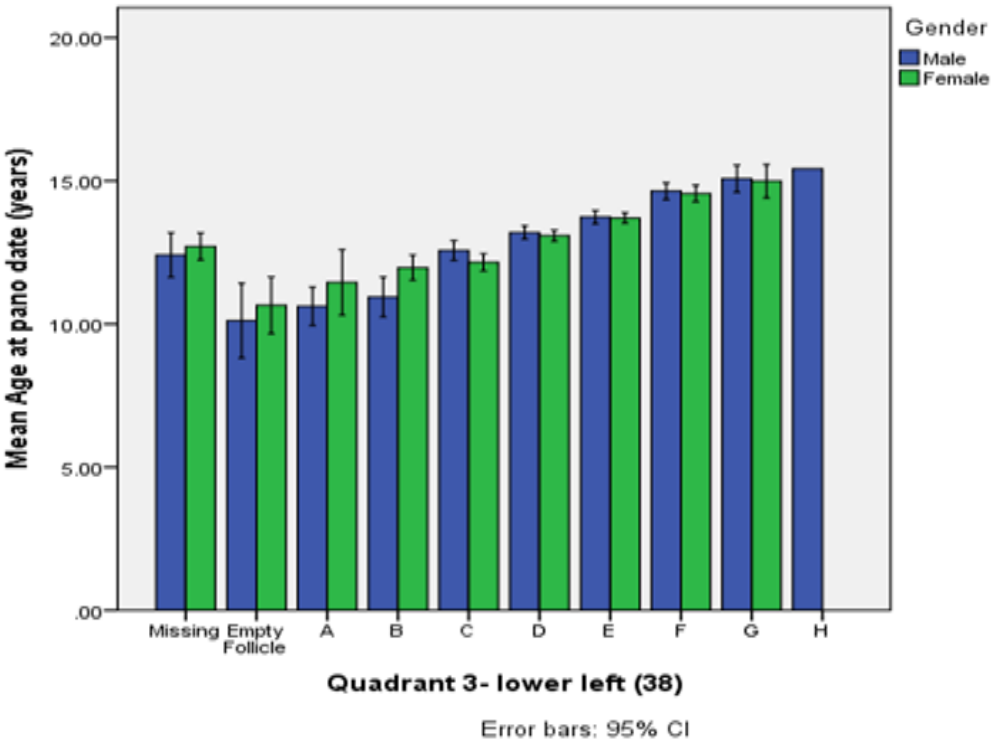


Figure 6: Mean age for all races in quadrant 3

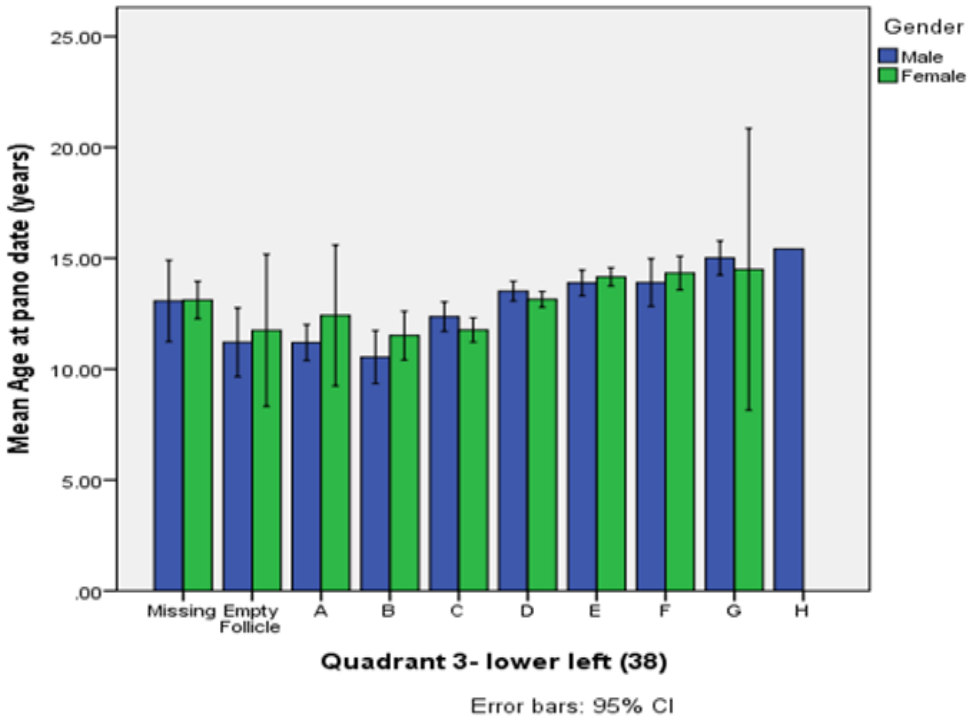


Figure 7: Mean age for Hispanics in quadrant 3

4.4.4 **Quadrant 4 Analysis**

Of 896 total subjects (mean age, 13.20 ± 1.56) years at Q4 (quadrant 4), 188 Hispanic subjects (mean age, 13.04 ± 1.59) years was the only race with enough sample for statistical comparisons. The mean age of males for all races (11.03 ± 1.68) years showed statistically significant lower mean than for females (11.95 ± 1.35) years on stage B, (p-value= 0.027). More specifically, Hispanic males (mean age, 10.22 ± 1.38) years showed statistically significant lower mean than females (mean age, 11.68 ± 0.84) years in stage B, (p-value= 0.021). Otherwise, there were no statistically significant mean differences in age between male and females on Q4 stages (p>0.05). Figures 8 and 9 illustrate the mean age of subjects at various stages for all races and solely Hispanics,

respectively.

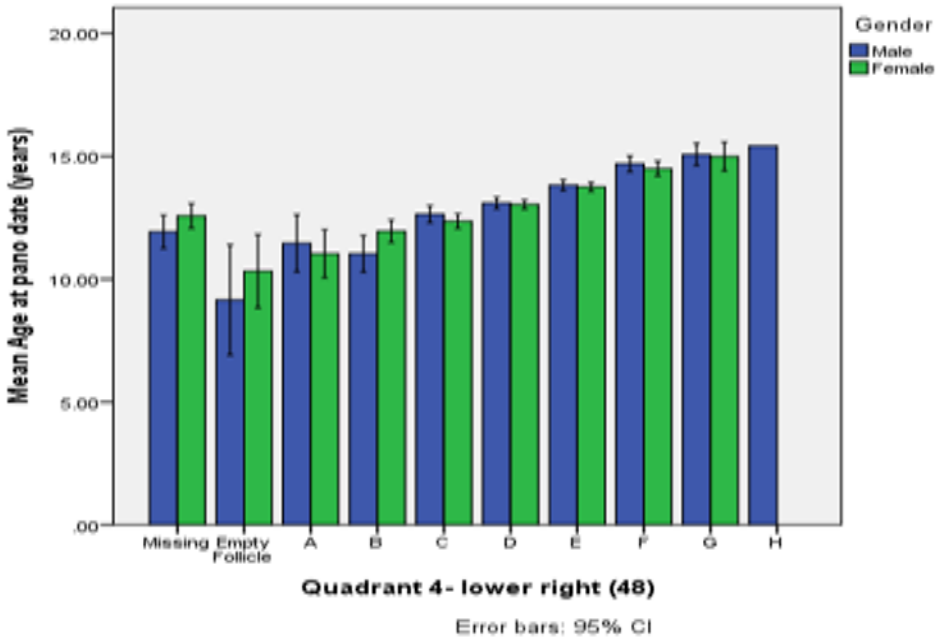


Figure 8: Mean age for all races in quadrant 4

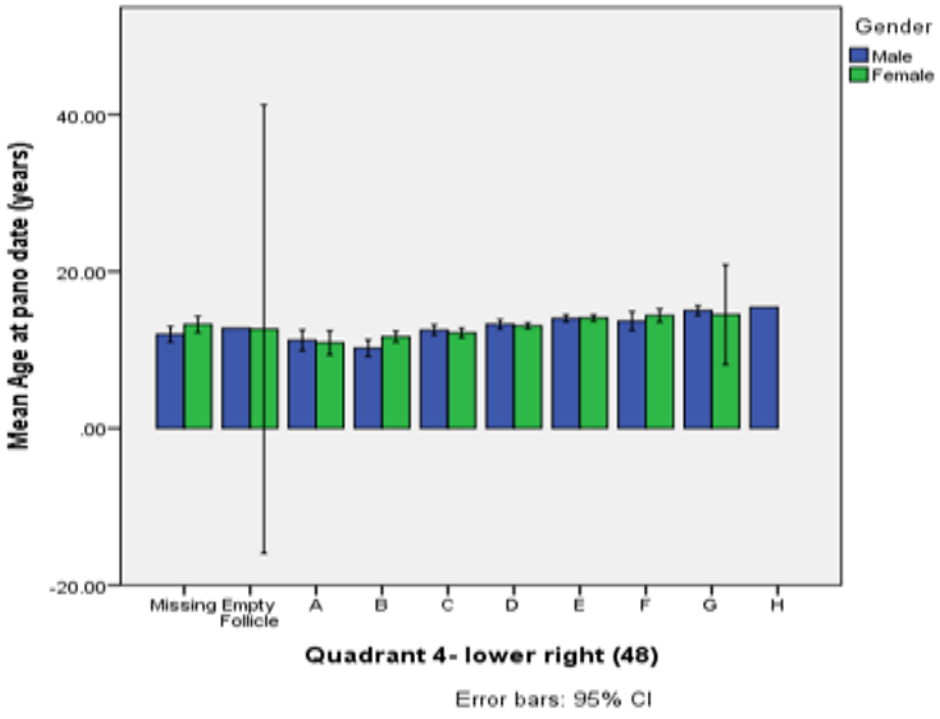


Figure 9: Mean age for Hispanics in quadrant 4

4.5 Comparison of Stages at Each Quadrant for All Races

The mean age, standard deviation, and 95% confidence interval for Q1, Q2, Q3, Q4 in all races are listed in tables IV-VII, respectively. There were no significant differences at all stages for all four quadrants, except for stage B in Q3 (p-value = 0.009) and stage B in Q4 (p-value = 0.027). Males in stage B Q3, had a mean age of 10.94 years with a standard deviation of 1.49 and a 95% confidence interval ranging from 10.25 to 11.64 years. In contrast, females at the same stage had a mean age of 11.97 years with a standard deviation of 1.19 and a 95% confidence interval ranging from 11.52 to 12.41 years. Males in stage B Q4, exhibited a mean age of 11.03 years with a standard deviation of 1.68 and a 95% confidence interval ranging from 10.29 to 11.78 years. On the other hand, females had a mean age of 11.95 years with a standard deviation of 1.35 and a 95% confidence interval ranging from 11.48 to 12.41 years. In both cases, development of third molars was earlier in males than females by 1.03 and 0.92 years, respectively. There were not enough subjects to record results at stage H for all quadrants and at the “empty follicle” stage for quadrants 1 and 2.

4.6 Comparison of Stages at Each Quadrant for Hispanics

The mean age, standard deviation, and 95% confidence interval for Q1, Q2, Q3, Q4 in Hispanics are listed in tables VIII-XI, respectively. There were no significant differences at all stages for all four quadrants, except for stage D in Q1 (p-value = 0.038) and stage B in Q4 (p-value = 0.021). Males in stage D Q1,

recorded mean age of 12.73 years with a standard deviation of 1.19 and a 95% confidence interval ranging from 12.08 to 13.39 years. In contrast, females at the same stage recorded mean age of 13.47 years with a standard deviation of 1.12 and a 95% confidence interval ranging from 13.11 to 13.82 years. Males in stage B Q4, recorded mean age of 10.22 years with a standard deviation of 1.39 and a 95% confidence interval ranging from 9.15 to 10.10 years. On the other hand, females recorded mean age of 11.68 years with a standard deviation of 0.84 and a 95% confidence interval ranging from 10.98 to 12.38 years. In both cases, stage development of third molars was earlier in males than females by 0.74 and 1.46 years, respectively. There were not enough subjects to record results at stage H for all quadrants, stages A, G for quadrants 1 and 2, and at the “empty follicle” stage for quadrants 1, 2 and 4.

TABLE IV

DESCRPTIVE STATISTICS AND INDEPENDENT STUDENT *T*-TESTS RESULTS (AGE IN YEARS) - QUADRANT 1 - (18)

Gender / Stages	Male					Female					*p-value
	N	Mean	SD	95% C I		N	Mean	SD	95% C I		
				Lower Bound	Upper Bound				Lower Bound	Upper Bound	
Missing Tooth	53	12.19	2.36	-	-	70	12.21	2.16	-	-	0.956
Empty Follicle	-	-	-	-	-	2	14.29	4.29	4.25	24.33	-
A	6	10.54	1.80	8.66	12.43	6	10.50	1.83	8.58	12.42	0.969
B	20	11.60	1.75	10.79	12.42	26	11.96	1.67	11.29	12.64	0.484
C	67	12.31	1.70	11.90	12.73	105	12.15	1.41	11.87	12.42	0.484
D	106	12.97	1.17	12.75	13.20	188	13.14	1.33	12.95	13.33	0.284
E	84	13.88	1.07	13.65	14.11	158	13.67	1.16	13.49	13.86	0.182
F	40	14.73	0.90	14.44	15.02	40	14.60	1.10	14.24	14.95	0.546
G	13	14.71	0.90	14.16	15.25	11	15.02	1.22	14.20	15.85	0.473
H	3	15.56	1.13	12.75	18.34	1	15.00	-	-	-	-

*Statistically Significant at 0.05.

TABLE V

DESCRPTIVE STATISTICS AND INDEPENDENT STUDENT *T*-TESTS RESULTS (AGE IN YEARS) - QUADRANT 2 - (28)

Gender / Stages	Male					Female					*p-value
	N	Mean	SD	95% C I		N	Mean	SD	95% C I		
				Lower Bound	Upper Bound				Lower Bound	Upper Bound	
Missing Tooth	56	12.33	2.42	-	-	71	12.00	2.05	-	-	0.400
Empty Follicle	-	-	-	-	-	2	13.08	4.24	-	-	-
A	2	9.63	1.23	7.93	11.33	4	10.50	1.11	8.73	12.27	0.429
B	20	11.56	1.85	10.69	12.42	22	11.63	1.50	10.96	12.30	0.891
C	68	12.24	1.71	11.83	12.66	114	12.20	1.38	11.94	12.45	0.843
D	116	13.06	1.22	12.84	13.28	185	13.17	1.35	12.97	13.36	0.486
E	76	13.79	1.03	13.55	14.02	155	13.75	1.13	13.58	13.94	0.827
F	39	14.74	0.88	14.46	15.03	42	14.58	1.08	14.24	14.91	0.451
G	12	14.74	0.93	14.14	15.33	11	15.02	1.22	14.20	15.85	0.533

*Statistically Significant at 0.05.

TABLE VI

DESCRPTIVE STATISTICS AND INDEPENDENT STUDENT *T*-TESTS RESULTS (AGE IN YEARS) - QUADRANT 3 - (38)

Gender / Stages	Male					Female					*p-value
	N	Mean	SD	95% C I		N	Mean	SD	95% C I		
				Lower Bound	Upper Bound				Lower Bound	Upper Bound	
Missing Tooth	35	12.41	2.26	-	-	73	12.71	2.01	-	-	0.493
Empty Follicle	12	10.12	2.05	8.81	11.42	17	10.65	1.92	9.66	11.64	0.479
A	13	10.62	1.11	9.94	11.29	11	11.46	1.71	10.31	12.60	0.161
B	20	10.94	1.49	10.25	11.64	30	11.97	1.19	11.52	12.41	0.009*
C	63	12.57	1.39	12.22	12.92	89	12.15	1.44	11.85	12.46	0.080
D	107	13.19	1.18	12.97	13.42	181	13.08	1.34	12.88	13.28	0.483
E	78	13.73	1.03	13.50	13.97	143	13.70	1.10	13.52	13.88	0.835
F	42	14.64	0.93	14.35	14.93	47	14.56	0.99	14.27	14.85	0.692
G	21	15.07	1.04	14.60	15.55	16	14.99	1.10	14.41	15.57	0.811
H	1	15.42	-	-	-	-	-	-	-	-	-

*Statistically Significant at 0.05.

TABLE VII

DESCRPTIVE STATISTICS AND INDEPENDENT STUDENT *T*-TESTS RESULTS (AGE IN YEARS) - QUADRANT 4 - (48)

Gender / Stages	Male					Female					*p-value
	N	Mean	SD	95% C I		N	Mean	SD	95% C I		
				Lower Bound	Upper Bound				Lower Bound	Upper Bound	
Missing Tooth	9	13.07	2.39	-	-	14	13.11	1.45	-	-	0.960
Empty Follicle	6	9.15	2.14	6.90	11.40	11	10.32	2.33	8.82	11.82	0.312
A	12	11.46	1.83	10.30	12.62	15	11.03	1.77	10.05	12.02	0.547
B	22	11.03	1.68	10.29	11.78	35	11.95	1.35	11.48	12.41	0.027*
C	66	12.64	1.41	12.29	12.99	90	12.36	1.45	12.06	12.66	0.226
D	101	13.09	1.17	12.87	13.32	189	13.04	1.33	12.85	13.23	0.729
E	85	13.82	1.02	13.60	14.04	140	13.76	1.11	13.57	13.94	0.651
F	37	14.69	0.96	14.37	15.00	48	14.50	1.04	14.20	14.80	0.390
G	22	15.07	1.02	14.62	15.52	16	14.99	1.10	14.41	15.57	0.814
H	1	15.42	-	-	-	-	-	-	-	-	-

*Statistically Significant at 0.05.

TABLE VIII

DESCRPTIVE STATISTICS AND INDEPENDENT STUDENT *T*-TESTS RESULTS IN HISPANICS (AGE IN YEARS) - QUADRANT 1 - (18)

Gender / Stages	Male					Female					*p-value
	N	Mean	SD	95% C I		N	Mean	SD	95% C I		
				Lower Bound	Upper Bound				Lower Bound	Upper Bound	
Missing Tooth	24	12.87	1.86	-	-	14	12.24	1.67	-	-	0.307
Empty Follicle	-	-	-	-	-	-	-	-	-	-	-
A	1	11.83	-	-	-	-	-	-	-	-	-
B	7	10.68	1.39	9.39	11.97	7	11.81	0.86	11.01	12.61	0.092
C	21	12.07	1.88	11.21	12.92	23	11.91	1.22	11.39	12.44	0.746
D	15	12.73	1.19	12.08	13.39	41	13.47	1.12	13.11	13.82	0.038*
E	16	13.81	1.00	13.28	14.34	27	13.90	1.07	13.48	14.32	0.787
F	6	14.40	0.71	13.66	15.15	6	14.37	1.42	12.88	15.87	0.966
G	3	14.81	0.54	13.47	16.14	-	-	-	-	-	-
H	1	15.42	-	-	-	1	15.00	-	-	-	-

*Statistically Significant at 0.05.

TABLE IX

DESCRPTIVE STATISTICS AND INDEPENDENT STUDENT *T*-TESTS RESULTS IN HISPANICS (AGE IN YEARS) - QUADRANT 2 - (28)

Gender / Stages	Male					Female					*p-value
	N	Mean	SD	95% C I		N	Mean	SD	95% C I		
				Lower Bound	Upper Bound				Lower Bound	Upper Bound	
Missing Tooth	19	12.85	2.15	-	-	16	12.44	1.66	-	-	0.540
Empty Follicle	-	-	-	-	-	-	-	-	-	-	-
A	-	-	-	-	-	1	10.83	-	-	-	-
B	9	11.31	1.74	9.97	12.65	5	11.60	1.00	10.36	12.84	0.742
C	23	12.22	1.73	11.47	12.97	24	12.01	1.12	11.54	12.48	0.627
D	23	13.05	1.44	12.42	13.67	40	13.46	1.19	13.08	13.84	0.222
E	11	13.58	0.96	12.93	14.22	25	13.88	1.07	13.43	14.32	0.427
F	6	14.40	0.71	13.66	15.15	7	14.32	1.31	13.11	15.53	0.894
G	3	15.19	1.35	11.84	18.55	1	15.00	-19	-	-	-
H	-	-	-	-	-	1	15.00	-	-	-	-

*Statistically Significant at 0.05.

TABLE X

DESCRPTIVE STATISTICS AND INDEPENDENT STUDENT *T*-TESTS RESULTS IN HISPANICS (AGE IN YEARS) - QUADRANT 3 - (38)

Gender / Stages	Male					Female					*p-value
	N	Mean	SD	95% C I		N	Mean	SD	95% C I		
				Lower Bound	Upper Bound				Lower Bound	Upper Bound	
Missing Tooth	9	13.07	2.39	-	-	14	13.11	1.45	-	-	0.960
Empty Follicle	4	11.21	0.98	9.66	12.76	4	11.75	2.15	8.32	15.18	0.664
A	8	11.20	0.97	10.39	12.00	2	12.42	0.35	9.24	15.60	0.130
B	9	10.55	1.55	9.35	11.74	7	11.51	1.19	10.42	12.61	0.194
C	20	12.37	1.42	11.70	13.03	20	11.77	1.17	11.22	12.31	0.154
D	18	13.52	0.90	13.07	13.97	36	13.15	1.05	12.80	13.50	0.209
E	13	13.89	0.95	13.31	14.47	24	14.16	0.98	13.74	14.57	0.431
F	6	13.90	1.03	12.82	14.98	10	14.33	1.06	13.58	15.09	0.441
G	6	15.01	0.73	14.24	15.78	2	14.50	0.71	12.54	16.46	0.422
H	1	15.42	-	-	-	-	-	-	-	-	-

*Statistically Significant at 0.05.

TABLE XI

DESCRPTIVE STATISTICS AND INDEPENDENT STUDENT *T*-TESTS RESULTS IN HISPANICS (AGE IN YEARS) - QUADRANT 4 - (48)

Gender / Stages	Male					Female					*p-value
	N	Mean	SD	95% C I		N	Mean	SD	95% C I		
				Lower Bound	Upper Bound				Lower Bound	Upper Bound	
Missing Tooth	40	11.93	2.11	-	-	63	12.57	1.20	-	-	0.121
Empty Follicle	1	12.75	-	-	-	2	12.67	3.18	-	-	-
A	4	11.23	0.83	9.90	12.55	5	10.90	1.24	9.36	12.44	0.666
B	9	10.22	1.39	9.15	10.10	8	11.68	0.84	10.98	12.38	0.021*
C	21	12.53	1.49	11.85	13.21	21	12.17	1.30	11.58	12.76	0.407
D	12	13.28	0.93	12.68	13.87	35	13.07	1.09	12.70	13.44	0.556
E	19	14.01	0.91	13.57	14.45	27	14.06	1.01	13.66	14.46	0.875
F	5	13.68	0.98	12.47	14.90	9	14.39	1.11	12.47	13.70	0.259
G	7	15.01	0.67	14.39	15.63	2	14.50	0.71	12.54	16.46	0.377
H	1	15.42	-	-	-	-	-	-	-	-	-

*Statistically Significant at 0.05.

5. DISCUSSION

5.1 Discussion

Age estimation through radiographic evaluation is one tool that could be used along with others to enhance diagnostic accuracy and improve identification of individuals. Several methods have been used to determine the most accurate way to estimate dental age. Demirjian et al. (1973), Gleiser and Hunt (1955), Moorrees et al. (1963), Gustafson and Koch (1974), Harris and Nortje (1984), Kullman et al. (1992), proposed methods that were tested in the past. In 2004, Olze et al. studied those methods, while concluding that the most accurate results were obtained with Demirjian et al.'s classification system due to providing a sufficient number of stages as well as defining them in lieu of length estimations. Demirjian et al.'s classification showed the highest values for both observer agreement and for correlation between the stages as defined by the method and true age (Olze et al., 2004). Demirjian presented a classification method differentiating eight stages of crown and root development, allowing for the evaluator to define stages by changes in shape with no metric estimations needed. Therefore, making it more objective and simple (Zeng et al., 2010). Panoramic radiographs improve the ability to enhance a wide view of the tooth and facial bones, making it one of the best instruments to assess dental structures (Meinl et al., 2007; Sisman et al. 2007).

In 2007, Sisman et al. studied third molar development in Turkish children and young adults, finding statistically significant differences between male and female in calcification stages D and G. In their study, third molars developed

earlier in males than females showing a strong correlation between age and third molar development for both sexes supporting the data presented in this study. In contrast to Sisman et al., the stages in which significant statistical differences occurred were in Q3 stage B and Q4 stage B when combining all races. More specifically, statistically significant differences in the Hispanic population occurred in Q1 stage D supporting the work of Sisman et al. (2007), but it was also shown in Q4 stage B which did not occur in their study.

In a study by Olze et al. (2004), third molar mineralization was evaluated in a Japanese population but no significant differences of their stage of development were found in the maxilla and mandible and between left and right sides, as shown in this study. According to Richardson et al. (1980), there's a 50% possibility of agenesis if the third molar has not appeared by the age of 10. The earliest evidence of third molar mineralization at stage A, was in Q1 in females at 10.50 ± 1.83 with a range from 8.58 -12.42 years of age. In 2003, Bolaños et al. recorded a higher proportion of agenesis of third molars in the maxilla. They later claimed that this could be due to poor visualization of maxillary third molar in relation to technological limitations, superposition or distortion of anatomical structures at the maxillary tuberosity in panoramic radiographs.

Complete crown formation at stage D ranged from 12.73 – 13.47 years of age in this study. Hence, it could be predicted that complete crown formation of third molars occurred around the age of 13, which was similar to a study conducted by Monirifard et al. (2015). There were not enough subjects to

determine mean age at stage H or complete radicular information, but the average age of stage G was between 14 and 15 years; hence it could be interpreted that radicular formation is completed around the age of 15 or slightly later. In comparison to Bolaños et al. (2003), crown formation completion occurred in this study only one year later than his findings, and radicular formation was completed three years after.

The method used in this study does not apply to subjects post completion of root formation. Once root formation is complete, it is difficult to determine when apices initially closed. In this case, investigators would not be able to fully determine an age range of an individual solely using this method. Most studies in the past have focused on radiographic images of tooth development to determine age in children and adolescents (Bolaños et al., 2003). According to Bolaños et al., after the age of 10 years, there is an increase in errors in the prediction of chronological age. Hence, age estimation through radiographic evaluation of third molar mineralization expands Bolaños et al.'s findings extending its application to include young adults.

The findings of this study were even more valuable in the younger population where stem cells have a higher potential to regenerate. Dental pulp stem cells (DPSCs) are mainly obtained from third molars, with previous studies showing that cells in the earlier stages of development have a better chance as a stem cell resource for tissue engineering (Seo et al., 2005; Horwitz et al., 2001). In 2014, Hadaegh et al. studied the pulp of human third molar teeth to find whether younger stem cell resources are more beneficial for future regenerative

purposes. They specifically looked at the presence of stem cells in the Nolla 6th stage, finding that they were vimentin positive proving their mesenchymal potential (Hadaegh et al., 2014). The cells expressed the markers CD73, CD90l CD105, CD166, and CD 144 with no expression of CD14, CD34, CD45, and HLA-DR further proving a possible niche for stem cell population being indicative of dental MSCs (Hadaegh et al., 2014). It is essential for stem cells to be able to proliferate and differentiate after cryopreservation, where they can be stored for future therapies depending on patients' needs. Seo et al. (2005) and Papaccio et al. (2006) both showed that the periodontal ligament and one month cryopreserved DPSCs respectively can maintain their potential as dental stem cells. DPSCs and their osteogenically differentiated cells after 2 years of cryopreservation are capable of proliferation and remodeling similarly as newly fresh cells (Woods et al., 2008). Hadaegh et al. (2014) proved that DPSCs isolated from the pulp tissue of wisdom teeth exhibit a higher proliferation rate when compared with bone marrow mesenchymal stromal cells in vitro due to its early stage of development with a higher potential to regenerate. Also, novel populations of MSCs from developing root of third molars have a higher potential to proliferate and differentiate when compared to mature pulp both in vivo and in vitro, showing that tissues at earlier stages of development could be a better resource for stem cell tissue engineering (Sonoyama et al., 2006; Jo et al., 2007). The ectomesenchymal soft tissue of third molars at N6th proves to be an ideal resource of highly potent stem cells as it could be obtained easily and safely due to the root not existing which could complicate the extraction process

(Hadaegh et al., 2014). In addition, cells at this stage express correct surface antigens and a high differentiation potential after long-term proliferation and cryopreservation making them an optimal resource for future bioengineering regenerative therapies (Hadaegh et al., 2014).

Third molars at stage N6th as defined by Nolla is where the development of crown is complete (Nolla et al. 1960), which translates into stage D according to Demirjian et al. (1973). According to the previous studies mentioned, stage D of third molar development would be the ideal stage in which these cells have a high potential for proliferation and differentiation. When clinically applicable, third molars at stage D or approximately the age of 13 years are best to excise for stem cell banking. When the donor's other teeth are missing, stem cells and tissue engineering technologies could permit the restoration of these missing teeth. This allows for the general dentist to be more aware of the age range acceptable to take a radiograph on a patient to further evaluate stem cell potential, leading to reduction in radiographic exposure.

5.2 Limitations of the Study

Out of 999 subjects, only 299 reported their ethnicity, thus, limiting full descriptive statistics on other races. Significant numbers on the Hispanic population were seen, but comparisons regarding various ethnicities in the same area could have given stronger conclusions in the pattern of age estimation. Illinois's Latino population ranked fifth in the nation in 2014 at 2.2 million people, with an annual average growth of 2.8 percent between 2007 and 2014 (Wong

and Moreno, 2016). Previous studies have reported differences between ethnic groups, but sufficient numbers are needed to evaluate results. Another limitation, was that images were taken by two panoramic machines from both departments (Orthodontic and Pediatric Dentistry), which could have resulted in slight differences in resolution/settings. Also, this study was not able to take into account other factors that may affect dental development including: genetics, climate, hormonal and environmental differences, and nutrition. Finally, the age range of the study was from 4-22, but there were not as many radiographs taken on patients younger than 7 years of age. This led to a low number of radiographs in the younger population of 4-7 years, where stage A or “empty follicle” were not available in some quadrants. Toward the opposite end of the classification spectrum, there were not enough subjects in stage H likely due to the fact that majority of subjects seen in the Orthodontic department at UIC range from 10-15 years of age.

5.3 Further Research

Additional studies with larger populations should be conducted to meet the need for population-based information on third molar development. So far, there is no consistent conclusion regarding how ethnicity influences the mineralization of the third molar with different studies showing different results (Zeng et al., 2010). Correlating age estimation with the stage of third molar development has shown to be inconsistent in the past among different races. Research in this subject will be beneficial to further evaluate ethnic differences in other

populations, as opposed to the UIC Orthodontic and Pediatric Dentistry Departments. This could improve the diagnostic accuracy when determining forensic age/identification of individuals, along with having a more valid speculation on the age of most asylum seekers.

AlQahtani et al. (2010) introduced the London Atlas of Human Tooth Development and Eruption as an alternate way to estimate age using both tooth development and alveolar eruption. It is still unclear whether this new method can replace the classic Demirjian method in terms of accurate age prediction as current published studies show conflicting results (AlQahtani et al., 2014; Alshiri et al. 2016). Further research is needed to test the London Atlas and its ability to precisely predict subjects' age through evaluation of their dental development.

The use of third molars as a source of stem cells could provide valuable resources for various conditions beneficial for future treatment of patients. Hence, it is essential that more research to be conducted in this field to further enhance the application of third molar stem cells. More investigations for further identification of DPSCs and in vivo studies are needed to further assess the regenerative potential in physiologic conditions before clinical application (Hadaegh et al., 2014).

6. CONCLUSION

In conclusion, there were no significant mean differences in the mineralization stages of the UIC population among the four quadrants. The male mean age of third molar mineralization was significantly earlier than that of females in Q3 stage B and Q4 stage B when combining all races. In the Hispanic population, third molar mineralization in male age was earlier than female age in Q1 stage D and Q4 stage B. Finally, it can be concluded that panoramic radiographic assessment of third molar mineralization is a useful tool for age estimation.

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APPENDIX A

Exemption Granted

September 8, 2015

Shehab Helal, DMD
Orthodontics
801 S Paulina St
Room 131, M/C 841
Chicago, IL 60612
Phone: (614) 578-3141 / Fax: (312) 996-0873

RE: Research Protocol # 2015-0892
"Age Estimation of Third Molar Mineralization Through Radiographic Evaluation"

Sponsors: None

Dear Dr. Helal:

Your Claim of Exemption was reviewed on September 8, 2015 and it was determined that your research protocol meets the criteria for exemption as defined in the U. S. Department of Health and Human Services Regulations for the Protection of Human Subjects [(45 CFR 46.101(b)]. You may now begin your research

Please note the following regarding your research:
Exemption Period: September 8, 2015- September 8, 2018
Performance Site: UIC
Number of Subjects: 2000
Subject Population: De-identified medical records initially collected for clinical purposes from January 1, 2005 through June 29, 2015.

The specific exemption category under 45 CFR 46.101(b) is:

(4) Research involving the collection or study of existing data, documents, records, pathological specimens, or diagnostic specimens, if these sources are publicly available or if the information is recorded by the investigator in such a manner that subjects cannot be identified, directly or through identifiers linked to the subjects.

HIPAA Waiver:
The Board determined that this research meets the regulatory requirements for waiver of authorization as permitted at 45CFR164.512 (i)(1)(i)(A). Specifically, that the use or disclosure of protected health information (PHI) meets the waiver criteria under 45CFR164.512 (i)(2)(ii); the research involves no more than a minimal risk to the privacy of the individuals; the research could not practicably be conducted without the waiver; and the research could not practicably be conducted without access to and use of the PHI.

You are reminded that investigators whose research involving human subjects is determined to be exempt from the federal regulations for the protection of human subjects still have responsibilities for the ethical conduct of the research under state law and UIC policy. Please be aware of the following UIC policies and responsibilities for investigators:

1. Amendments You are responsible for reporting any amendments to your research protocol that may affect the determination of the exemption and may result in your research no longer being eligible for the exemption that has been granted.

2. Record Keeping You are responsible for maintaining a copy all research related records in a secure location in the event future verification is necessary, at a minimum these documents include: the research protocol, the claim of exemption application, all questionnaires, survey instruments, interview questions and/or data collection instruments associated with this research protocol, recruiting or advertising materials, any consent forms or information sheets given to subjects, or any other pertinent documents.

3. Final Report When you have completed work on your research protocol, you should submit a final report to the Office for Protection of Research Subjects (OPRS).

Please be sure to:

→Use your research protocol number (2015-0892) on any documents or correspondence with the IRB concerning your research protocol.

We wish you the best as you conduct your research. If you have any questions or need further help, please contact me at (312) 996-1711 or the OPRS office at (312) 413-0241. Please send any correspondence about this protocol to OPRS at 203 AOB, M/C 672.

Sincerely,

Ibraheem Oguntade

IRB Coordinator, IRB # 2

Office for the Protection of Research Subjects

cc: Carlotta A. Evans, Orthodontics, M/C 841
Maria Therese S. Galang, Orthodontics, M/C 841
Privacy Office, Health information management Department, M/C 772

Exemption Determination
Amendment to Research Protocol – Exempt Review
UIC Amendment #1

December 9, 2016

Shehab Helal, DMD
Orthodontics
801 S Paulina St
Room 131, M/C 841
Chicago, IL 60612
Phone: (614) 578-3141 / Fax: (312) 996-0873

RE: Protocol # 2015-0892
"Age Estimation Through Radiographic Evaluation of Third Molar Mineralization"

Dear Dr. Helal:

The OPRS staff/members of Institutional Review Board (IRB) #7 have reviewed and approved this amendment to your research, and have determined that your amended research continues to meet the criteria for exemption as defined in the U. S. Department of Health and Human Services Regulations for the Protection of Human Subjects [(45 CFR 46.101(b)].

The specific exemption category under 45 CFR 46.101(b) is:
(4) Research involving the collection or study of existing data, documents, records, pathological specimens, or diagnostic specimens, if these sources are publicly available or if the information is recorded by the investigator in such a manner that subjects cannot be identified, directly or through identifiers linked to the subjects.

You may now implement the amendment in your research.

Please note the following information about your approved amendment:
Exemption Period: **December 9, 2016 – December 9, 2019**
Amendment Approval Date: December 9, 2016
Amendment:

Summary: UIC Amendment #1: Changing research title from "Age Estimation of Third Molar Mineralization Through Radiographic Evaluation" to "Age Estimation Through Radiographic Evaluation of Third Molar Mineralization"

You are reminded that investigators whose research involving human subjects is determined to be exempt from the federal regulations for the protection of human subjects still have responsibilities for the ethical conduct of the research under state law and UIC policy. Please be aware of the following UIC policies and responsibilities for investigators:

1. Amendments You are responsible for reporting any amendments to your research protocol that may affect the determination of the exemption and may result in your research no longer being eligible for the exemption that has been granted.

2. Record Keeping You are responsible for maintaining a copy all research related records in a secure location in the event future verification is necessary, at a minimum these documents include: the research protocol, the claim of exemption application, all questionnaires, survey instruments, interview questions and/or data collection instruments associated with this research protocol, recruiting or advertising materials, any consent forms or information sheets given to subjects, or any other pertinent documents.

3. Final Report When you have completed work on your research protocol, you should submit a final report to the Office for Protection of Research Subjects (OPRS).

Please be sure to use your research protocol number (2015-0892) on any documents or correspondence with the IRB concerning your research protocol.

We wish you the best as you conduct your research. If you have any questions or need further help, please contact me at (312) 355-2908 or the OPRS office at (312) 996-1711. Please send any correspondence about this protocol to OPRS at 203 AOB, M/C 672.

Sincerely,

Charles W. Hoehne, B.S., C.I.P.

Assistant Director, IRB #7
Office for the Protection of Research Subjects

cc: Carlotta A. Evans, Orthodontics, M/C 841
Maria Therese S. Galang-Boquiren, Orthodontics, M/C 841

VITA

Shehab Ossama Helal

EDUCATION:
Certificate in Orthodontics, and Master of Science in Oral Sciences, University of Illinois at Chicago, Department of Orthodontics, Chicago, IL, Anticipated 2014-2017

DMD, University of Kentucky Dental School, Lexington, KY, 2014

Bachelor of Arts, Miami University, Oxford, OH, 2010 Major: Zoology, Minor: Economics

EXPERIENCE:
Orthodontic Teaching Assistant, University of Illinois at Chicago College of Dentistry, Chicago, IL, 2014 – present

Research Investigator, University of Kentucky College of Dentistry, Orthodontics Department, Lexington, KY, 2012 -2014

Research Assistant, Miami University, Botany Summer Scholars Program, Oxford, OH, 2009

AWARDS AND HONORS:
- Interprofessional Deans' Honors –UKCD 2014
- University of Kentucky College of Dentistry Enhancement Scholarship-UKCD 2014
- Dean's List- Miami University 2010
- National Honor Society of Collegiate Scholars Certificates – Miami University 2007
- Botany Awards Scholar – Miami University 2010

ORGANIZATION/MEMBERSHIP ACTIVITIES:
- American Association of Orthodontics (AAO) 2014-present
- Delta Sigma Delta (DSD dental fraternity) 2012-2014
  -Class representative/ Senior page 2012-2014
- American Student Dental Association (ASDA) 2010-2014
  -Website Editor 2012-2013
- Student National Dental Association (SNDA) 2010-2014
- Student Research Group 2010-2014
  -Newsletter Editor 2012-2013
- Student Professional Ethics Association (SPEA) 2012-2014
  -Treasurer 2012-2013
- American Association of Women Dentists (AAWD) 2012-2014