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SECTION AGE ESTIMATION

# The variability of lower third molar development in Northeast Malaysian population with application to age estimation

N.A. Johan[1], M.F. Khamis[1], N.Sk. Abdul Jamal[2], B. Ahmad[1], E.S. Mahanani[3]

[1] *School of Dental Sciences, Health Campus, Universiti Sains Malaysia 16150 Kubang Kerian, Kelantan, Malaysia.*
[2] *Kulim Main Dental Clinic, Jalan Hospital Lama, 09000 Kulim Kedah Malaysia*
[3] *Dentistry study program, Faculty of Medicine and Health Science, Muhammadiyah University of Yogyakarta, Jl. Lingkar Selatan, Tamantirto, Kasihan, Yogyakarta 55183, Indonesia*

***Corresponding author:*** *fadhli@kb.usm.my*

*The authors declare that they have no conflict of interest.*

***ABSTRACT***

***This study aimed to assess the variability of the lower third molar (tooth 38 and 48) development in Northeast Malaysian population with respect to the side of dentition, to generate age prediction models and to compare the outcome with other studies. A total of 1080 orthopantomograms of Northeast Malaysian population aged between 14 and 25 years (540 males and 540 females) from the Hospital Universiti Sains Malaysia's archive which met the inclusion and exclusion criteria were selected and the maturity stages of toothth 38 and 48 were scored using Demirjian's stages (A-H). The findings showed a wide variation of the development of lower third molars in the Northeast Malaysian population. The roots developed earlier in males than in females. The development of the dentition on opposite sides of the mandible was synchronously in females and males. A multiple regression analysis shows that 71.1% of variance in age was explained by sex and developmental stage of tooth 48. An age prediction model was generated from the regression analysis: [Age = 7.117 + 1.907*(stage of tooth 48) – 0.432*(sex)] with mean prediction errors between -0.17 to 3.14 years. The obtained data in the current study are useful for references and determining age of unidentified human remains for identification investigation.***

***KEYWORDS***: Third molar development, Age estimation, Multiple linear regression, Northeast Malaysian, Demirjian's stages

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## *INTRODUCTION*

Age estimation is useful for human identification[1] and in determining legal age for criminal responsibility.[2] Both applications are related to local legal requirements and can be applied to ageing both human remains and living people.[3]

Dental evidence is of great importance for forensic age estimation procedures as teeth are the most durable structure of the human body[4] and they give results of acceptable accuracy for assigning the age.[5] Moreover, dental post-mortem data can predict the age of a person from approximately 18-20 weeks 'in utero' until the last tooth is lost.[6]

Dental age can be evaluated in young children with higher accuracy because many teeth are undergoing development and mineralization simultaneously.[7] However, most of teeth have completed their development by approximately 14 years old,[8] leaving only the third molars to continue maturing until a later age.

The third molars are variable teeth in terms of position, size, shape, timing of formation and eruption and agenesis,[9] nevertheless, many studies found that the third molar development was applicable for age estimation.[2, 10-14]

There is no specific trend of sexual differences in the third molar development was reported across several populations and geographical areas. Studies on American Whites,[10] Texas Hispanic,[15] Europeans such as Belgian,[16] Spanish,[11] Turkish[17] and Austrian[18] and several Eastern Asians[2, 14, 19] reported that the third molar development is more advanced in males than in females. Few reverse findings are reported among North Indian[12] and American Black.[10]

Since there is no information regarding to age estimation in Northeast Malaysian population, the present study aimed to assess the variability of the lower third molar (tooth 38 and 48) development in Northeast Malaysian population aged between 14 to 25 years in both sexes, with respect to the side of dentition, and to generate an age prediction model. The accuracy and the regression analyses were also compared with other publications.

## *MATERIALS AND METHODS*

### *Materials*

Orthopantomograms (OPG) from the Hospital Universiti Sains Malaysia's archive were screened. Poor quality OPGs and those subjects with obvious dental pathology, known history of chronic medical illness and hormonal deficiency were all excluded. Non locals, based on the information from their new registration identification card number in the dental records, were also excluded.

All OPGs had been taken using Orthoralix 9200 (Finland) with different xray doses depended on the patient's body size, 70kV (small), 74kV (medium) and 78kV (large). The distance between subject and X-ray source was set at 0-14mm (default=7mm) and the exposure was 12 seconds. The study was granted ethical clearance to access dental records by the Universiti Sains Malaysia Human Ethics Committee. The handling of dental records complied with the highest standard of ethics.

Based on sample size calculation, a total of 1080 orthopantomograms of Northeast Malaysian population aged between 14 and 25 years (540 males and 540 females) were required. The calculation of the sample size was based on these parameters: the mean difference of 1.5 years with 80% power and alpha 0.05 which consists of 45 subjects in each study group (45 x 12 age

groups x sex). Standard deviation was estimated as 2.5 years.[18]

***Methods***

The mineralization of both mandibular third molars was assessed according to the method described in Demirjian *et al*[5] using the eight grade scheme where stages A to D described the crown formation, while stages E to H describe root development.[5] Intra and inter-observer reliability was tested by re-examining 30 OPGs after a week interval.

***Statistical analysis***

Intra and inter-observer reliability were analyzed using Kappa Agreement.[20] Mean ages with standard deviation at each developmental stage were calculated and analyzed. Sex differences in age of attainment were tested using independent t-test. Side differences of age of attainment were assessed by Spearman '*rho*' correlation coefficient. A prediction model and its accuracy were tested using stepwise regression method in a multiple linear regression analysis. The stepwise method criteria used probability of *F* (entry of 0.05 and removal of 1.0). All statistical analyses were performed using PASW 18.0[21] and significance level was set at 0.05.

**Results**

Intra-observer reliability was excellent with a high value of agreement of 0.96 ($p<0.001$) for both sides of third molars. Inter-examiner reliability was also as high as 0.84 ($p<0.001$) for tooth 38 and 0.88 ($p<0.001$) for tooth 48.

Tables 1 and 2 show the distribution and the frequency of Demirjian's stages of lower third molars (tooth 38 and 48) according to chronological age group and for both sexes. In this sample the root apex for tooth 38 begins to close (Stage H) at age 18 years for both sexes. However, for tooth 48 one female case reached stage H at 17 years old.

***DISCUSSION***

The intra- and inter-observer reliability were high so it is unlikely that they biased the results and interpretation of our study.[22] This supports the popularity of Demirjian's atlas approach which is not dependent on the length of root or any metric measurement. The present study focused on the lower third molars but not the maxillary third molars due to the difficulty in assessing the latter teeth reliably as adjacent structures on the orthopantomograms (e.g. floor or posterior wall of maxillary sinus, zygomatic arch) superimpose the maxillary teeth.[2] One female subject reached complete root development before the age of 18 years old. Thus, this may limit the forensic use of the third molar development for estimating legal age. However, further study with larger sample size at stages development of G and H would be required before this conclusion can be made.

The results of this study showed that the development of third molars in Northeast Malaysians was more advanced in males than in females. The root development in males was ahead of females by more than 6 months; while the sexual dimorphism was less obvious in crown development, where the difference was less than 5 months. These findings are consistent with other Eastern Asians (0.2 - 1.5 years)[2, 14] and Europeans (0.2 – 1.49 years).[23, 24]

Multiple regression analysis demonstrated the level of association between chronological age, the tooth development stages and sexes. As development stage of tooth 38 and 48 were strongly correlated, a

**Table 1:** Frequency of Demirjian's stage of left third molar

| Chronological age groups (years) | Demirjian's stages | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Females | | | | | | | Males | | | | | | |
| | C | D | E | | G | H | Total | C | D | E | F | G | H | Total |
| **14** | 11 | 27 | 7 | | | | 45 | 12 | 23 | 9 | 1 | | | 45 |
| **15** | 7 | 18 | 14 | 5 | 1 | | 45 | 4 | 13 | 19 | 8 | 1 | | 45 |
| **16** | 2 | 15 | 19 | 8 | 1 | | 45 | 3 | 8 | 14 | 14 | 6 | | 45 |
| **17** | | 5 | 12 | 19 | 9 | | 45 | | 3 | 8 | 15 | 19 | | 45 |
| **18** | | 3 | 6 | 13 | 16 | 7 | 45 | | | 4 | 11 | 21 | 9 | 45 |
| **19** | | 1 | 1 | 18 | 14 | 11 | 45 | | | 4 | 6 | 21 | 14 | 45 |
| **20** | | | | 4 | 23 | 18 | 45 | | | | 4 | 15 | 26 | 45 |
| **21** | | | | 2 | 22 | 21 | 45 | | | | | 9 | 36 | 45 |
| **22** | | | | 1 | 12 | 31 | 45 | | | | | 5 | 40 | 45 |
| **23** | | | | | 9 | 36 | 45 | | | | | 5 | 40 | 45 |
| **24** | | | | | 5 | 40 | 45 | | | | | 2 | 43 | 45 |
| **25** | | | | | 1 | 44 | 45 | | | | | 1 | 44 | 45 |
| **Total** | 20 | 69 | 59 | 70 | 114 | 208 | 540 | 19 | 47 | 58 | 59 | 105 | 252 | 540 |

multicollinearity problem was detected (*VIF*>10). Thus the analysis was completed using tooth 48 and sex only. Previous publications[12, 16, 17] generate separate/unique sex prediction models, however, our study included sex into the model since the interaction between sex and tooth development did not contributet significantly to the model ($p>0.05$). This allows the unique contribution of each tooth's development and sex to age being quantified. Thus, one prediction model can be used to predict age for either males or females among Northeast Malaysians.

Every scientific evidence should have a sound methodology, known error rate and acceptabilityby the scientific community to be admissible in court.[25] Thus, our study provided the error rates and SD. The average prediction error is less than a year except for few age groups, in which the predicted error was up to 3.14 years. The SD values were also comparable with other publications for Belgian Caucasian and Thail population.[16, 26] So far, there is no study reporting the mean error and the SD values of each age group for comparisons with our study.

The regression model should be used cautiously when the stage of tooth has reached stage H. The prediction model is limited to the predicted ages of 22.37 years for females and 21.94 years for males, as the highest value of "8" (stage H) was

**Table 2:** Frequency of Demirjian's stage of right third molar

| Chronological age groups (years) | Demirjian's stages | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Females | | | | | | | Males | | | | | | |
| | C | D | E | F | G | H | Total | C | D | E | F | G | H | Total |
| **14** | 13 | 22 | 10 | | | | 45 | 12 | 25 | 7 | 1 | | | 45 |
| **15** | 8 | 15 | 18 | 3 | 1 | | 45 | 4 | 17 | 16 | 6 | 2 | | 45 |
| **16** | 3 | 12 | 21 | 6 | 3 | | 45 | 3 | 5 | 18 | 14 | 5 | | 45 |
| **17** | | 5 | 13 | 20 | 6 | 1 | 45 | | 3 | 6 | 18 | 18 | | 45 |
| **18** | | 2 | 7 | 12 | 19 | 5 | 45 | | | 4 | 13 | 23 | 5 | 45 |
| **19** | | | 2 | 18 | 17 | 8 | 45 | | | 2 | 8 | 21 | 14 | 45 |
| **20** | | 1 | | 4 | 25 | 15 | 45 | | | | 3 | 17 | 25 | 45 |
| **21** | | | | 2 | 24 | 19 | 45 | | | | 1 | 13 | 31 | 45 |
| **22** | | | | 1 | 11 | 33 | 45 | | | | | 7 | 38 | 45 |
| **23** | | | | | 7 | 38 | 45 | | | | | 5 | 40 | 45 |
| **24** | | | | | 6 | 39 | 45 | | | | | 2 | 43 | 45 |
| **25** | | | | | 2 | 43 | 45 | | | | | 1 | 44 | 45 |
| **Total** | 24 | 57 | 71 | 66 | 121 | 201 | 540 | 19 | 50 | 53 | 64 | 114 | 240 | 540 |

considered for the developmental stage. This explains why the error (between observed and predicted age) was increased in older age group and always under-predicted. Other methods of dental age estimation should be considered for cases with stage H such as pulp size changes[27] and estimation of aspartic acid racemization in dentine.[28]

The trend of $R^2$ values of our study was comparable with other Asian populations but higher than reported $R^2$ values for European ancestry. This conclusion can be made since the scoring method was also Demirjian *et al*.[5] method. The value of $R^2$ 0.711 is considered high and perhaps better than the skeletal age.[29]

## *CONCLUSION*

A wide variability exists in the development of third molar teeth. Correlation between sides generally strong, and the third molar root developed earlier in males than in females. The age

**Table 3:** Descriptive statistics and sex differences in chronological age of left third molars development for males and females

| Demirjian's stage | Females | | | Males | | | Mean diff | 95% CI | t-statistics | df | p value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | Mean (years) | SD | n | Mean (years) | SD | | | | | |
| **Stage C** | 20 | 14.55 | 0.686 | 19 | 14.53 | 0.772 | 0.024 | -0.45,0.50 | 0.10 | 35.0 | 0.920 |
| **Stage D** | 69 | 15.16 | 1.232 | 47 | 14.81 | 0.947 | 0.351 | -0.05,0.75 | 1.73 | 112.3 | 0.086 |
| **Stage E** | 69 | 15.98 | 1.225 | 58 | 15.84 | 1.142 | 0.138 | -0.35,0.62 | 0.57 | 112.2 | 0.573 |
| **Stage F** | 70 | 17.80 | 1.528 | 59 | 17.03 | 1.474 | 0.766 | 0.24,1.29 | 2.89 | 124.7 | ***0.005*** |
| **Stage G** | 114 | 20.15 | 2.011 | 105 | 19.03 | 2.031 | 1.129 | 0.59,1.67 | 4.13 | 215.2 | ***<0.001*** |
| **Stage H** | 208 | 22.63 | 1.984 | 252 | 22.37 | 1.980 | 0.260 | -0.11,0.62 | 1.40 | 441.3 | 0.162 |

SD, standard deviation; n, sample size; mean diff, mean difference; CI, confidence interval; df, degree of freedom

**Table 4:** Descriptive statistics and sex differences in chronological age of right third molars development for males and females

| Demirjian's stage | Females | | | Males | | | Mean diff | 95% CI | t-statistics | df | p value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | Mean (years) | SD | n | Mean (years) | SD | | | | | |
| **Stage C** | 24 | 14.58 | 0.717 | 19 | 14.53 | 0.772 | 0.057 | -0.41,0.52 | 0.25 | 37.3 | 0.805 |
| **Stage D** | 57 | 15.19 | 1.302 | 50 | 14.72 | 0.882 | 0.473 | 0.05,0.90 | 2.22 | 99.0 | ***0.028*** |
| **Stage E** | 71 | 15.93 | 1.291 | 53 | 15.81 | 1.257 | 0.118 | -0.34,0.58 | 0.51 | 113.8 | 0.609 |
| **Stage F** | 66 | 17.92 | 1.460 | 64 | 17.20 | 1.449 | 0.721 | 0.22,1.27 | 2.83 | 128.0 | ***0.005*** |
| **Stage G** | 121 | 20.09 | 2.033 | 114 | 19.16 | 2.033 | 0.933 | 0.41,1.46 | 3.52 | 232.2 | ***0.001*** |
| **Stage H** | 201 | 22.73 | 1.910 | 240 | 22.48 | 1.930 | 0.252 | -0.11,0.61 | 1.37 | 427.0 | 0.170 |

SD, standard deviation; n, sample size; mean diff, mean difference; CI, confidence interval; df, degree of freedom

prediction model for the Northeast Malaysians *i.e.* 7.117 + 1.907(stage of development) – 0.432(sex) is applicable for age prediction with the averages SD 1.82 years and 1.90 years for females and males respectively. However, this prediction model should be cautiously applied when the stages of the tooth development has reached stage H. The applicability of this model is also limited to stages C to H due to the design of the study and statistical analyses. Further study on younger age group (stages A and B) and focusing on legal age would be beneficial.

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**Table 5:** Relationship of tooth developmental stages between sides in both sexes

| Sex | Correlation coefficient | p |
|---|---|---|
| **Females** | 0.936 | <0.01 |
| **Males** | 0.915 | <0.01 |

**Table 6:** Un-standardized coefficients of multiple regression analysis[a]

| Predictors | Unstandardized Coefficients | | t-statistics | p value | 95% Confidence Interval for *B* | |
|---|---|---|---|---|---|---|
| | *B* | Std. Error | | | Lower Bound | Upper Bound |
| **(Constant)** | 7.117 | 0.253 | 28.098 | <0.001 | 6.620 | 7.613 |
| **Stage of development at right third molar** | 1.907 | 0.037 | 51.537 | <0.001 | 1.834 | 1.980 |
| **Sex** | -0.432 | 0.114 | -3.802 | <0.001 | -.655 | -0.209 |

[a] Lower left third molar (tooth 38) was omitted due to multicollinearity *VIF*>10

**Table 7:** Mean of prediction error (observed – predicted) and standard deviation for sex and chronological age

| Chronological age groups (years) | Females | | Males | |
|---|---|---|---|---|
| | Mean prediction error (years) | Standard deviation | Mean prediction error (years) | Standard deviation |
| **14** | -0.58 | 1.36 | -0.17 | 1.28 |
| **15** | -0.53 | 1.76 | -0.68 | 1.84 |
| **16** | -0.42 | 1.84 | -0.80 | 1.93 |
| **17** | -0.87 | 1.80 | -1.41 | 1.75 |
| **18** | -1.38 | 1.94 | -1.35 | 1.59 |
| **19** | -0.89 | 1.64 | -1.06 | 1.59 |
| **20** | -0.74 | 1.47 | -1.03 | 1.18 |
| **21** | -0.22 | 1.03 | -0.31 | 0.98 |
| **22** | 0.13 | 1.00 | 0.32 | 0.79 |
| **23** | 0.91 | 0.73 | 1.25 | 0.58 |
| **24** | 1.89 | 0.70 | 2.10 | 0.45 |
| **25** | 2.67 | 0.63 | 3.14 | 0.47 |
| **average** | | 1.82 | | 1.90 |

Negative sign indicates over-prediction (observed – predicted); Codes used for prediction model: Stage of development 1 to 8 equation to A to H; sexes 0=female, 1=male; example of model prediction for stage H, female: Age of prediction = 7.117 + 1.907(8) -0.432(0)=22.37 years. If males (code=1), the predicted age is equivalent to 21.94 years.

**Table 8: S**ummation of multiple linear regression analysis results on third molar development from different studies.

| Population/study | $R^2$ | Age prediction model/regression formula | SD(years) |
|---|---|---|---|
| **Northeast malaysian** | **0.711** | **age = 7.117 + 1.907(stage of tooth 48) – 0.432(sex) whole sample and sex variable was included** | **refer to table 7** |
| **Belgian caucasian[16]** | **0.37 (f) 0.48 (m)** | **separate male and female** | **1.56 (f) 1.52 (m)** |
| **Turkish[17]** | **0.61 (f) 0.57 (m)** | **separate male, female and whole sample** | **n/a** |
| **Thai[26]** | **n/a** | **separate male and female, and specific for each jaw** | **1.71-1.90** |
| **North indian[12]** | **0.63 (f) 0.62 (m)** | **separate male, female and whole sample** | **n/a** |
| **Spanish[23]** | **0.45 (f) 0.54 (m)** | **n/a** | **n/a** |
| **Turkish[24]** | **0.56 (f) 0.57 (m)** | **n/a** | **n/a** |
| **Korean[2]** | **0.81 (f) 0.84 (m)** | **n/a** | **n/a** |
| **Romanian[30]** | **0.94 (f) 0.95 (m)** | **n/a** | **n/a** |

(F) Females; (M) Males; SD Standard deviations; all predictions models and coefficients of determination are within 95% CI. N/A data were not available

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