VALIDATION OF THIRD MOLAR MATURITY INDEX (I3M) FOR DISCRIMINATION OF JUVENILE / ADULT STATUS IN SOUTH INDIAN POPULATION

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Abstract*:*

Deliberate falsification of age was considered to be one of the main reasons for forensic age estimation of the living individuals. This posed to be a challenging task during criminal and legal proceedings, and ultimate care must be taken not to classify juveniles as adults. Third molars are the only developing teeth during late adolescence and early adulthood. Our study was designed to analyze the usefulness of the third molar maturity index (I_{3M}) specific cut-off value (I_{3M} <0.08) to discriminate adults (≥ 18 years) and juveniles (<18 years) in South Indian children.

216 panoramic radiographs (114 females and 102 males) of living subjects aged between 14 and 21 years were analyzed. Our results demonstrated high sensitivity (83.3% and 90.2%) and specificity (98.3 % and 95.1%) for females and males respectively. The positive likelihood ratios of being adult were 50.00 and 18.35 while the negative likelihood ratios were 0.17 and 0.10 in females and males respectively. The estimated posttest probability was 98.0% in females and 94.8% in males. The obtained results showed that the specific cut-off value of I_{3M} <0.08 may be a useful additional tool in discrimination of individuals who are around 18 years of age.

Keywords: Forensic Sciences, Forensic Anthropology, Third molar maturity index, South Indian population, Panoramic radiographs

1. *Introduction*:

Age estimation of individuals requires a multidisciplinary approach and predicting individual's attainment of the age of majority is of primary importance in many cases[1-3]. The age of majority is the age at which the law considers someone reached adulthood and proclaimed to

be a full legal citizen who further doesn't require supervision of a parent or guardian in decision making[4, 5]. Assessment of biological age in late adolescent and early adult individuals, around the legal cut-off age of 18 years, has become a challenge for forensic experts[6]. Comprehensive age estimation in investigations will utilize all available methods and development of third molars with further compliments of the skeletal indicators may give an assessment of the age of unknown individual within expected confidence interval[7]. Applicability of third molars in age estimation was previously reported and tested in practice; however, some authors mark them as unreliable indicators, because of the different presence, malposition, and different time of initial formation and the wide age range of mineralization[8, 9]. On the other hand, the review of medical and anthropology literature evinced undisputed usefulness on third molar development for age assessments in subadult individuals[10-13]. The process of apical closure of permanent teeth, excluding third molars, finishes between the age of 12 to 14 years and after that third molars are the only immature teeth available for age estimation in preadolescents and early adolescents. Radiographic analysis of third molars expands the years of age estimation from 9 to 23 years, and their initiation, development, and eruption are closely related with age[14, 15].

Estimation of the age of an individual may become necessary in some circumstances, and virtually no age is immune from medico-legal scrutiny[16]. Given reality, when an undocumented individual has taken to penal and criminal justice, it is critical to determine whether the individual is an adult or juvenile. The age of criminal culpability vary among countries and are dealt with by the juvenile justice systems. According to Section 2 (aa) of the Indian immoral traffic (prevention) Act, 1956, a "child" is defined as a person who has not completed 16 years of age, in addition According to Section 2 (cb) a "minor" is a person who has completed 16 years of age but has not completed the age of 18[17].

The minimum age of criminal responsibility (MACR) is the age below which a person is completely immune from any criminal liability due to lack of maturity and judgment to understand the consequences of one's actions. In India, the criminal system is governed and regulated by two major legislations including the Indian Penal Code, 1860 (IPC) and the Criminal Procedure Code, 1970 (CrPC). The IPC has set the minimum age of criminal responsibility as 12 years[18]. The Juvenile Justice (Care and Protection of Children) Act, 2000 is legislation that confirms to the United Nations minimum standards for administration of justice to children and as per this legislation children cannot be put into the same category as adults and

hence required to develop special provisions for them[19]. This act has set the age of criminal responsibility at 18 years that concurs definition of child under the UN convention on the rights of the child[18].

The determination of adult or juvenile is a legal question, and not a scientific one, and it is the responsibility of forensic professionals to provide age estimation reports based on reliable scientific methods. In the case of living individuals, third molar maturity is likely to be the best suitable method as it is low-invasive in nature and can be evaluated on radiograph[7]. Mincer et al. [20] were the first to study the usefulness of the third molars to discriminate juvenile versus adult status of the evaluated individuals[20]. Cameriere et al.[21] have demonstrated the better performance of I_{3M} <0.08 in discriminating adults or juveniles when compared to Demirjian staging (DS) system. The latter was successfully applied in various populations and proven to be a successful method in predicting the age of majority[22-28].

A sample of South Indian adolescents and adults was evaluated in this study. Up to date, no studies have validated the applicability of Cameriere's third molar maturity index in the South Indians. Therefore, the main aim of this study was to test the usefulness of Cameriere's cut-off value of I_{3M} <0.08 in discrimination adults and juveniles of the evaluated individuals.

2. Material and methods

2.1 Sample

Digital panoramic radiographs (OPTs) of 216 living South Indian subjects, aged between 14 and 21 years, were analyzed retrospectively (Table 1). The OPTs utilized in this study belongs to the healthy individuals who visited Panineeya Institute of Dental Sciences, Hyderabad, India. These OPTs were taken as a routine pretreatment dental examination. Approval for the usage of these OPTs was obtained from the Institutional Ethical Committee for research involving human subjects. The subject's details were preserved and each OPT was assigned an identification number. Chronological age (in years) and sex were recorded separately in an Excel file. The chronological age of each subject was calculated as the difference between the date of exposure of the OPT and the date of birth and converted into decimal ages. The inclusion criteria were: subjects between 14 and 21 years, those with known age, good quality radiographs and without medical evidence of systemic diseases which can affect growth

including diabetes, hypothyroidism, hormonal therapy and poor nutrition or intestinal diseases. Individuals with unknown birth dates and those with missing third molars, severe caries, fillings, or with developmental anomalies that may affect measurements on third molars, were excluded.

2.2 Measurements

The selected digital radiographs were saved in JPEG format. To adjust a gray scale, brightness and contrast, image quality improvement tools in Adobe® Photoshop® CS4 were used. The FDI (Fédération Dentaire Internationale) two-digit system notation of the teeth was used. The left mandibular third molars (TMs) were assessed according to the method of Cameriere et al.[21]. Since the development of teeth "No.38" and "No.48" is symmetric and strongly correlated, multicollinearity problems in the regression models could be detected[29, 30]. Therefore, for standardization, and according to the original study by Cameriere et al.[21], only TMs from the left side of the mandible were evaluated, i.e. tooth "No.38" [31-33]. The apical ends of the roots of the left lower third molar of each were analyzed, and the measurements were performed using a computerized image-processing program (ImageJ) [34].

Briefly, I_{3M} was defined as follows: if the root development of the third molar is complete, i.e., the apical ends of the roots are completely closed, then I_{3M} =0.0, otherwise I_{3M} is evaluated as the sum of the distances between the inner sides of the two open apices (A_i, i = 1, . . ., 7) divided by the tooth length (L_i, i = 1, . . ., 7). I_{3M} is evaluated in a similar way to the ratio A_i to L_i, when i = 6 or 7, as reported for the first and second lower molars in Cameriere et al.[35]. Determination of I_{3M} allows the use of a single predicting variable which is achieved by normalizing the values of the width of the apices and height of the teeth.

2.3 Statistical analysis

Each OPT was coded with a numerical ID so as to prevent observer bias, and the observer, therefore, was not aware of the age or sex of the subjects. The age of each was calculated as the difference between the x-ray day collection and the patient's birthday.

To assess the intra-rater and inter-rater agreement of I_{3M}, intraclass correlation coefficient (ICC)[36] was calculated three weeks after the first measurements on 30 individuals randomly

sampled[21]. All the analyses were performed using a blind approach with the readers not aware of the sex and age of the patients.

Analysis of covariance (ANCOVA) was conducted to study possible interaction between real age, I_{3M} and sex. The I_{3M} and the sex of the subjects were used as the predictive variable for age estimation. The correlation between age and third molar index (I_{3M}) was tested with Pearson's correlation coefficient.

Cameriere et al.[21] recommended the same cut-off value of I_{3M} <0.08, for both sexes, that an individual is considered to be 18 years of age or older. The two-by-two contingency tables were used to list the performance of the test. The test has given the true results if those who are 18 years and more have I_{3M} <0.08 (true positives, TP) or negative if those who are under 18 years have I_{3M} ≥0.08 (true negatives, TN). Additionally, the test is misleading if those who are under 18 years have I_{3M} <0.08 (false positive, FP) and finally if those who are 18 years and more have I_{3M} ≥0.08 (false negative, FN)[25]. The sensitivity of the test, *p1* (i.e.: the proportion of the subjects 18 years and older who have I_{3M} <0.08), together with the specificity *p2* (i.e.: the proportion of individuals younger than 18 who have I_{3M} ≥0.08) were evaluated. The positive likelihood ratio (LR+) and negative likelihood ratio (LR-) were also calculated. Likelihood ratios in our study express how many times more or less likely a test result is to be found in adults compared with juvenile participants[37]. The post-test probability, *p*, of being 18 years of age or older can help to discriminate between those individuals who are 18 and over and under 18.

According to Bayes' theorem, post-test probability may be written as:

$$p = \frac{p1p0}{p1p0+(1-p2)(1-p0)} \qquad (1)$$

Where *p* is post-test probability and *p0* is the probability that the subject in question is 18 years old or older, given that he or she is aged between 14 and 21 years, which represent the target population. Probability *p0* was calculated as the proportion of India between 18 and 21 years of age who live in the South India according to demographic data from the 2011 census (http://www.censusindia.gov.in/2011census/C-series/C-13.html) and those between 14 and 21 years which was evaluated from data from the same web source. This proportion was considered

to be 0.50 both for boys and girls. All statistical analyses were performed using the IBM SPSS 22.0 software program (IBM® SPSS® Statistics, Armonk, NY). The significant threshold was set at 5% and 1% as reported in the text.

3. Results

The intra- and inter-observer agreement were ICC =98.8% (95% CI, 97.0% -99.5%) and ICC =94.6% (95% CI, 88.2% - 97.5%).

In this study, carried out on 216 healthy Indian subjects a minimum of 21 (17 and 18 years) and a maximum of 40 (15 years) individuals were studied per age and sex (Table 1). ANCOVA showed no interaction between I_{3M} and sex to real age ($p > 0.05$). Sample scores of I_{3M} range from 0.00 to 2.1 depending on the age group as detailed in Figure 1. Distribution of real age gradually decreased as I_{3M} increased, in both females and males (Fig. 1). The relationship between the age of the subjects and I_{3M} is presented in Figure 2. The mean ages in each I_{3M} class varied between sexes (Table 2) but the differences were not statistically significant ($p > 0.05$). Correlation between the I_{3M} and the age is statistically significant and negative, ($r = -0.754$, $p < 0.001$) in females and $r = -0.706$, $p < 0.001$) in males.

Although no differences in sexes were detected, the performance of the cut-off value of $I_{3M} < 0.08$, reported in Cameriere et al.[21], was tested on the contemporary South Indian sample, separately on females and males [38].

The results of the analysis of the effectiveness of $I_{3M} < 0.08$ were presented in two two-by-two contingency tables (Tables 3a, b). Table 3a shows the close association between adult age and the positivity of the test ($I_{3M} < 0.08$) in females. Of 114 individuals, 104 were accurately classified or 91.2% (95%CI, 86.0%-96.4%). The sensitivity of the test for females was 83.3% (95% CI, 73.4%-93.3%) and the specificity was 98.3% (95% CI, 95.1%-100.0%).

Table 3b shows the close association between adult age and the positivity of the test ($I_{3M} < 0.08$) in males. Totally 95 out of 102 individuals were accurately classified or 93.1% (95% CI,

88.2%-98.0%). The sensitivity of the test (the proportion of individuals being 18 years of age or older whose test was positive) was 90.2% (95% CI, 81.2%-99.3%) and the specificity of the test (the proportion of individuals younger than 18 years whose test was negative) was 95.1% (95% CI, 89.7%-100.0%).

Positive likelihood ratios (LRs+) were 50.00 (95% CI, 7.13-350.47) and 18.35 (95% CI, 6.06-55.57) while negative likelihood ratio (LRs-) were 0.17 (95% CI, 0.09-0.31) and 0.10 (95%CI, 0.04-0.26) in females and males respectively. Estimated post-test probabilities were 98.0% (95% CI, 89.0%-100.0%) and 94.8% (95% CI, 85.4%-100.0%) in females and males respectively.

4. Discussion

Deliberate falsification of age for various purposes is considered to be one of many reasons for forensic age estimation of the living individuals[39]. A wide variety of methods based on the skeletal maturity[40-44] and dental development[31, 45, 46] have been published for age estimation. All these methods have proven to be accurate when applied to the individuals from the population from which those standards are derived[47]. It is a known fact that the application of foreign standards to the testing population results in a proportionate reduction of the expected accuracy. This has become a constant challenge for the forensic practitioners. At prior, it is important to assess the levels of accuracy of these foreign standards and the degree of dissimilarity between the original reference sample and to those for whom these standards are applied[48, 49]. Age estimation in living thus needed to be performed using appropriate population-specific standards[47].

According to our knowledge, this is the first study which used OPTs to test the accuracy of Cameriere's third molar maturity index cut-off value of I_{3M} <0.08 in discriminating juveniles and adult status on South Indian adolescents and young adults. India itself is a great and composite country where the southern regions display great diversity in religions, cultures, languages and vast socioeconomic disparities. Illegal migration of individuals without proper documentation was considered as one of many reasons to estimate the age and his/her attainment of the age of majority.

Cameriere et al.[21] presented a method to assess the age of majority, which is based on the relation between real age and the proportions of widths of open apices and the tooth length of

third molars. Later the specific cut-off value of I_{3M} <0.08 has been tested for different populations[22-28], which further confirmed its applicability and reliability.

In our study, the same cut- off value (I_{3M} <0.08) was applied for validation of South Indian population. It demonstrated good sensitivity and specificity values, comparable with previous studies on I_{3M} <0.08[1, 22].

Both sexes showed better specificity, 98.3% in females and 95.1% in males, than sensitivity, 83.3% in females and 90.2% in males. Males were better classified (93.1%) than females (91.2%) between adults and juveniles. Our findings are comparable to the most studies on the usefulness of I_{3M} <0.08 in discriminating adults and minors in different populations[1, 6, 22-28, 50, 51]. The most recent Libyan study, by Dardouri et al.[50], showed some better performance of the test, specificity was 100.0% in both sexes with sensitivity of 90.6% and 90.9% and accurate classification of 94.5% and 95.1% in females and males respectively while performance in our study was better than in Australian study, by Franklin et al.[22], they showed sensitivity of 90% in both sexes, specificity of 88% and 85% and accurate classification of 88% and 87% in females and males respectively.

The intra- and inter-observer agreements calculated as ICC were excellent, which showed the uniformity and reproducibility of the applied I_{3M} method.

Our study has demonstrated some earlier maturation of males over females in all I_{3M} classes of maturation of lower left TM (Figure 1), the mean ages for males were lower across all I_{3M} classes (Table 2), but the differences were not statistically significant (p >0.05), which is in line with some previous studies on I_{3M}[21, 26].

In the context of Indian legal system, the assessment of an individual's age is crucial because of increased involvement of children and adolescents in committing crimes[17]. Previously, several authors studied the application of DS system and another approach for estimating the age, including the age of majority[20, 52-61]. For estimating the age of majority, Mincer et al..[20], showed the low accuracy if DS was used. Several authors also found that large percent of individuals would be incorrectly classified as non-adults with DS method[28].

Acharya[62] was the first in Indian context to use DS approach to discriminate juvenile/adult status and reported that one in four cases resulted in "incorrect classification" which he believed as an insufficient level of accuracy for the courts to adapt. Later, Acharya et al.[63] applied the grading system of Köhler et al.[64] to assess the ability of third molars in

determining the age of majority, and summarized that only 35–37% of the sample examined falls into "reliable" prediction of juvenile/adult status, which is just over one in three cases. Based on the allocation accuracies, the author also suggested that Köhler's grading of third molars in Indians may be disadvantageous to individuals <18 years old, because of its tendency in the wrongful prediction of a juvenile as an adult.

Age estimation using teeth was studied in India widely[65-68]. Recently, a study was conducted on South Indian children, where in which the author has tested the accuracy of three age estimation methods[65]. Despite its slight underestimation of real age, Cameriere's method[69] was proved to be the best method over Willems[70] and Acharya's method[62]. Further affirmative to the results, this study is designed to evaluate the applicability of I_{3M} for discrimination of juvenile/adult status on South Indian subjects. The present study exploited the specific value of the proportion of the projections of open apices and height of third molars on OPTs and attempted to verify their accuracy for assessment of the age of majority in South Indian subjects. Till date, no study has evaluated the applicability of I_{3M} <0.08 for discrimination of juvenile/adult status on Indian subjects.

It has been emphasized in literature, that each of the parameters (such as ethnicity, hereditary, climatic conditions, nutrition, etc.) that are influencing the development and mineralization of tooth must be taken into consideration. Many studies over the recent years tested the effect of population and ethnicity on mineralization of third molars came up with different results[71, 72].On another hand, some recent findings suggested that the ethnical or national differences in third molar development are of clinically minor effect[73]. In 2008, Cameriere et al.[21] presented a method to assess the age of majority, which is based on the relation between real age and the proportions of widths of open apices and the tooth length of third molars. Later the specific cut-off value of I_{3M} <0.08 has been tested for different populations[22-28], which further confirmed its applicability and reliability.

Predicting one's attainment of the majority age is posed as a challenging task in forensic practice. This difficulty even reflects during the process the decision making for judges. In forensic field, what interests the judges is whether the questioned individual has reached a specific threshold to classify her or him as a juvenile or as a major[74]. In the legal point of view, it

is important to minimize the proportion of errors during discrimination of juvenile/adult status of individuals. These errors are separated as technically unacceptable (adults mistakenly classified into non-adults) and ethically unacceptable errors (juveniles incorrectly classified in the adult group)[25, 26]. In our study, first type error occurred in 9 out of 54 females and 4 out of 41 females, while 1 of the 60 females and 3 of 61 male non-adults had shown second type error. This slightly increased number of incorrectly classified adult females corresponds to the delayed development of the third molars when compared to males[28]. When it comes to an assessment of age in preadolescents, utmost importance must be given not to classify juveniles as adults, since it may lead to unfair treatment of these children in society or institutions and also to many violations such as legal rights, the right to asylum etc.[26]. Particular attention must be given to the methods utilized and also have to make sure whether these applied standards are enhancing the accuracy of forensic age estimates or not. It is also important to extend the study of age estimation to different reliable methods as suggested under the guidelines by Study Group on Forensic Age Diagnostics of the German Society of Legal Medicine (AGFAD)[75]. Also considering the facts of existing differences even within the same country, the authors emphasize the importance of extending this study to other regions of India (North Indian) to investigate possible regional variability.

5. Conclusion:

In conclusion, the findings of this study demonstrated that Cameriere's cut off value (I_{3M} <0.08) is adequate to discriminate juvenile/adult status in South Indian population, especially when a test with high credibility, including specificity and accuracy, is required.

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Figure 1 Boxplot of relationship between age and third molar maturity index of the mandibular left third molar in South Indian females and males. Boxplot shows median and inter-quartile ranges while whiskers are lines extending from box to maximum and minimum ages, including outliers.

Figure 2 Scatter-plot of the relationship between age (years) and the third molar maturity index (I_{3M}) in South Indian females and males.

List of tables:

Table 1 Sample of panoramic radiographs according to sex and age categories.

Age (years)	Females	Males	Total
14	14	21	35
15	21	19	40
16	14	11	25
17	11	10	21
18	10	11	21
19	15	10	25
20	13	10	23
21	16	10	26
Total	114	102	216

Table 2 Summary statistics of chronological age according to third molar maturity index (I_{3M}) classes

I_{3M} classes	N	Mean	SD	Min	Med	Max
Females						
[0.0, 0.04)	34	20.58	1.25	18.13	20.71	21.99
[0.04, 0.08)	12	19.28	1.02	18.89	19.16	21.92
[0.08, 0.3)	32	17.21	1.64	14.26	16.88	21.02
[0.3, 0.7)	15	16.04	1.17	14.47	15.68	19.39

[0.7, 2.1]	21	15.08	0.92	14.07	14.58	17.70
Males						
[0.0, 0.04)	33	20.13	1.20	17.73	20.27	21.94
[0.04, 0.08)	7	18.59	0.67	17.52	18.82	19.43
[0.08, 0.3)	25	16.90	1.61	14.87	16.43	21.47
[0.3, 0.7)	17	15.40	0.76	14.22	15.41	17.01
[0.7, 2.1]	20	14.88	0.68	14.01	14.68	16.42

Abbreviation: N, number of individuals , Mean, mean age; SD, standard deviation of mean age; Min, Minimal age; Med, median age; Max, maximum age

Table 3 Contingency tables describing discrimination performance between adults (≥18 years) and juveniles (<18 years) of the cut-off value of third molar maturity index (I_{3M} <0.08) for females and males

a) Females				**b) Males**			
Test	≥18	<18	Total	Test	≥18	<18	Total
<0.08	45^{TP}	1^{FP}	46	<0.08	37^{TP}	3^{FP}	40
≥0.08	9^{FN}	59^{TN}	68	≥0.08	4^{FN}	58^{TN}	62
Total females	54	60	114	Total males	41	61	102

Abbreviation: TP, true positives; FP, false positives; FN, false negatives; TN, true negatives

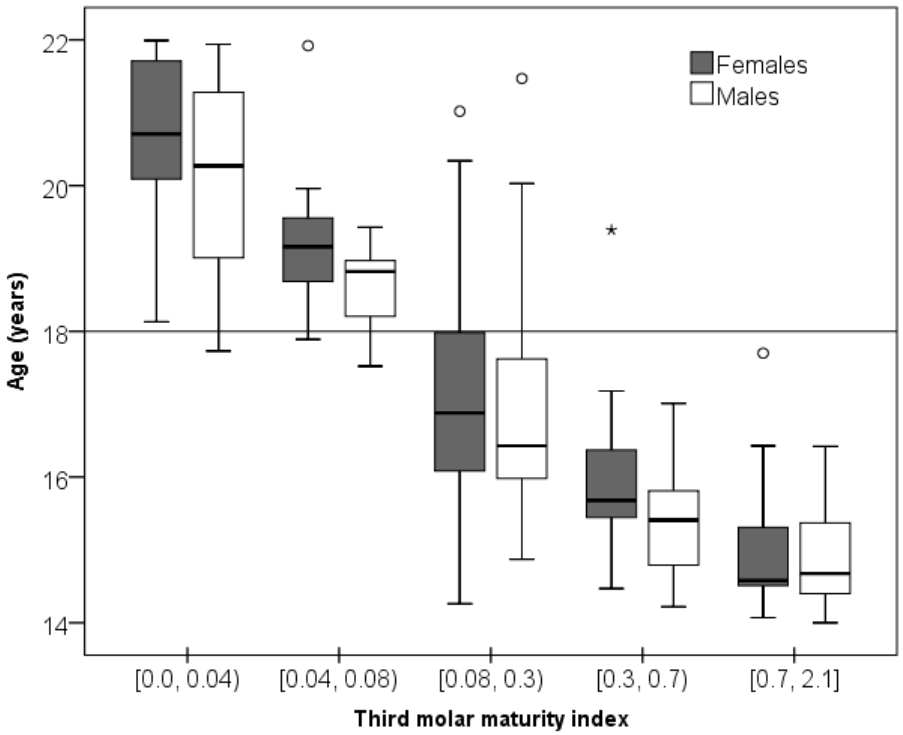

Figure 1 Boxplot of relationship between age and third molar maturity index of the mandibular left third molar in South Indian females and males. Boxplot shows median and inter-quartile ranges while whiskers are lines extending from box to maximum and minimum ages, including outliers.

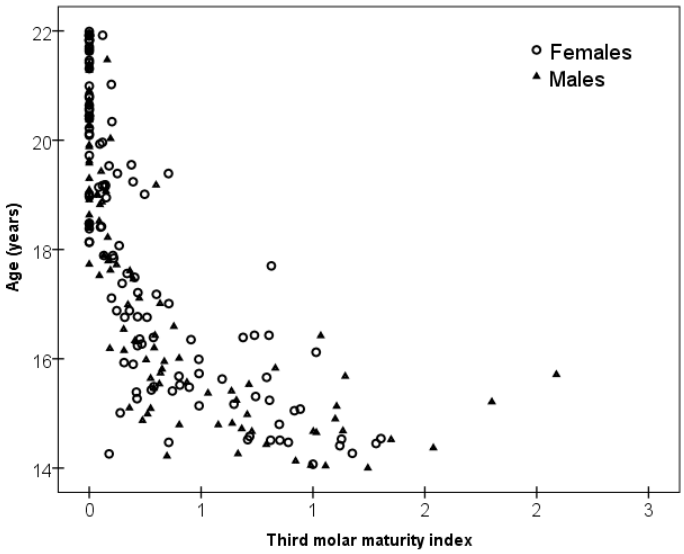

Figure 2 Scatter-plot of the relationship between age (years) and the third molar maturity index (I_{3M}) in South Indian females and males.