Evaluation of third molar maturity index (I3M) in assessing the legal age of subjects in an Indian Goan population

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ABSTRACT

India affords special laws and exemptions to minors under the criminal, marriage, labour and administrative laws. Many perpetrators claim to be a minor in the hope of a lenient trial and verdict. The authorities often rely upon forensic experts to provide evidence-based reports. The third molar can be relied upon in the assessment of legal age as it continues developing into the early twenties. The method established by Cameriere et al in 2008 provides an objective method for the accurate evaluation of legal age. Our study was designed to analyze and validate the efficacy of Third Molar Maturity index (I3M) in an Indian Goan population and compare it to published literature. 542 panoramic radiographs of subjects aged between 14 and 24 years were evaluated. The chronologic age increased as I3M reduced. There was no evidence of sexual dimorphism in third molar development across various I3M classes (p>0.05). Receiver Operator Characteristic Curve was plotted for males and females which showed an Area Under Curve of 0.95 (95% CI, 0.92-0.97) and 0.93 (95% CI, 0.90-0.96) respectively. 2x2 contingency tables were used to test the performance of various I3M cut-off values ranging from I3M=0.02 to 0.14. I3M = 0.08 showed the most promising results for the assessment of legal age. Our study achieved a high degree of accurate classification of 0.90 and 0.88 for males and females respectively. Results demonstrate a sensitivity of 0.899 and 0.854 and specificity of 0.90 and 0.93 for males and females respectively. The positive likelihood ratios were 9.88 and 12.44 while negative likelihood ratio was 0.11 and 0.15 for males and females respectively. A favourable Bayes Post Test Probability of 0.95 was noted for both males and females. These results allow us to strongly recommend the use of I3M for the assessment of legal age in an Indian Goan population.

INTRODUCTION

It is common to find undocumented minors facing civil or criminal charges. In all cases of suspicion and in accordance with Indian criminal, marriage, and administrative laws, the prosecutor or corresponding administrative authority is obliged to establish the age of the purported minor[1]. This necessitates accurate and reliable estimation of legal adulthood of a subject. The application of the most widely used age estimation techniques such as Demirjan's[2] and Willems[3] in assessment of legal adulthood have some drawbacks. They are subjective, rely on evaluation of parameters influenced by environmental and nutritional factors or provide an age range as opposed to an

accurate age. Thus, these approaches may not be applicable while attempting to assess legal adulthood.

Dental age estimation using the third molars could be ideal for estimation of legal age as they are the last teeth to develop and they can offer information with regard to this critical age[4].

Cameriere's third molar maturity index (I3M) offers a technique which uses objective analysis of the third molar development to assess legal age[5]. This potentially overcomes the drawbacks previously stated. The method uses the ratio of inter-pulpal distance between the open apices of the third molar and the height of the third molar. A threshold (cut-off) value of 0.08 was identified and used to discriminate between individuals who are above or below legal age.

Multiple studies have been published in recent literature evaluating the efficacy of the I3M cut-off of 0.08 in assessing legal age of 18 in their populations[6,8-20]. It has been established that the development of the dentition can vary with race[21]. Thus, it is important to verify the third molar maturity index of the local population and to compare them to published literature from other populations. The aim of this study was to evaluate the cut-off value of I3M=0.08 previously recommended by Cameriere in an Indian Goan population.

MATERIAL AND METHODS

Sample Collection
Panoramic radiographs (OPGs) of 576 Indians between 14 – 24 years of age were collected from the database of the Department of Oral Medicine and Radiology, Goa Dental College. The inclusion criteria ensured images of good quality, complete patient records and of known age (14 – 24 years) when the OPG was obtained. The samples with missing third molars, incomplete records, developmental anomaly or pathology involvement were excluded. The final sample size used for evaluation was 542. (Table 1)

The OPG number, chronologic age, gender and date of radiography was recorded using Microsoft Excel while maintaining anonymity of the patient. Institutional Research Ethical Committee approval was grated and the study was conducted in accordance with the ethical standards of the Declaration of Helsinki.

Table 1. Number of samples collected within various age groups where number in parenthesis represents samples with closed apices of Left Third Molar (I3M=0)

Number of Samples for I3M			
Age (Years)	**Males**	**Females**	**Total**
14 – 14.9	35	27	**62**
15 – 15.9	31	22	**53**
16 – 16.9	31	29	**60**
17 – 17.9	24 (3)	24 (3)	**48**
18 – 18.9	33 (14)	29 (8)	**62**
19 – 19.9	33 (19)	30 (12)	**63**
20 – 20.9	31 (28)	34 (21)	**65**
21 – 21.9	28 (23)	31 (23)	**59**
22-24	23 (20)	47 (41)	**70**
Total	**269**	**273**	**542**

Measurements
The sample was obtained and stored as a JPEG. The chronologic age of the subject was calculated by subtracting the date of birth from the date on which OPG was obtained. Adobe Photoshop CS7 was used to adjust colour, contrast, grey scale to improve the quality of image and to carry out the measurement of I3M. The left third molar of males and females was evaluated separately in accordance with previously published literature for the purpose of standardization. I3M was determined as previously described by Cameriere et al.[3] Briefly, if the third molar has root development complete, i.e., apical ends of the roots completely closed, then I3M = 0, otherwise, I3M is evaluated as the inter-pulpal distance (A) divided by the tooth length (L). In case of a multi-rooted tooth the inter-pulpal distance is measured at the apex as the sum of the distances between the inner sides of each root (A1 + A2). Both impacted and non-impacted third molars were included in the study.

Statistical Analysis and Data Management
SPSS Statistics 22.0 for Windows (IBM® SPSS®) and MS Excel 2016 (Microsoft® Office 2016) were used for all statistical analyses and data

management. All measurements and calculation of I3M were performed after blinding of the OPG records by the first observer. Inter-observer and intra-observer reliability was calculated using Inter-class Correlation Coefficient (ICC) on 56 random OPG samples (10% total sample size) to assess the correlation between the first and second observers respectively. To calculate intra-observer error, the first observer repeated the measurements of OPGs after one month of recording the first set of observations. Mann Whitney U test was performed to evaluate sexual dimorphism within I3M classes.

A Receiver Operator Characteristic Curve (ROC Curve) was used to assess the reliability of the test. It is a graphic representation of the relationship between sensitivity (Se) and specificity (Sp) in a test. The curve is confined in a unit square. The left-lower corner (Se = 0, Sp = 1) corresponds to the highest possible test cut-off value. As the cut-off value decreases, the test Se increases and Sp decreases, moving on the curve from the left-lower corner up and to the right to ultimately reach the right-upper corner of the square where Se = 1 and Sp = 0, corresponding to the lowest possible test cut-off value. The area under the curve (AUC) is indicative of the reliability of the test. An AUC of 0.5 would mean a worthless test, on the other hand an AUC of 1 depicts a perfect test.[21] A 2x2 contingency table was plotted to test the performance of other I3M cut-off levels. The results showed the number of participants who were 18 and above with I3M < the cut-off value, those who were less than 18 years with I3M ≥ cut-off value, those who were under 18 with I3M < the cut-off and those who were older than 18 with I3M ≥ the cut-off. A participant who is 18 and above with I3M< cut-off value was defined to have a positive test.

Using the 2x2 contingency table, measurements for Accurately Classified (AC) individuals, the Sensitivity (Se), Specificity (Sp), Youden's Index (J-Index), Positive Predictive Value (PPV), Negative Predictive Value (NPV), Positive Likelihood Ratio (LR+), Negative Likelihood Ratio (LR-) and Bayes Post Test Probability (Bayes PTP) was calculated for various cut-off values. J-index is a function of the Se and Sp and captures the performance of a diagnostic test. The PPV is the proportion of the participants who were true positives. The NPV is the proportion of the participants who were true negatives. The LR+ is the ratio of the true

positive rate (sensitivity) to the false positive rate (1 −Se). This likelihood ratio statistic measures the value of the test for increasing certainty about a positive diagnosis. The LR- is the ratio of the false negative rate to the true negative rate (Sp). The likelihood ratios indicate how many times more or less likely adults are to have I3M<0.08 than minors and minors are to have I3M≥0.08 than majors.

Bayes Post Test Probability (Bayes PTP) may be written as

$$ P = \frac{P1P0}{(1 - P2)(1 - P0) + P1P0} $$

where P is post-test probability, P1 is Sensitivity, P2 is Specificity and P0 is the probability that the participant in question is 18 years or older given that he or she is aged between 14 and 24 years, which represents the target population. In this study, probability P0 was calculated as the proportion of participants between 18 and 24 years of age and those between 14 and 24 years who live in Goa. This data was obtained from the 2011 census for India. The P0 was found to be 0.65 and 0.63 in males and females respectively.

RESULTS

The mean age of the total sample among males and females was found to be 18.31 and 19.01 years respectively. The first case of apical closure was noted at the age of 17 years for males and females. As expected, the number of cases showing apical closure increased steadily as the age increased (Table 1). It was observed that a greater proportion of males achieved apical closure at a younger age compared to females (Table 1). The highest I3M value noted was 1.48. A box plot showing the relationship between chronologic age and I3M demonstrated that the I3M ratios increased as the mean ages decreased. (Figure 1)

The ICC results yielded 0.992 (95% CI, 0.987 to 0.996) and 0.994 (95% CI, 0.987 to 0.997) for the intra-observer and inter-observer tests respectively. There was no sexual dimorphism observed within the I3M classes (Table 2).

The ROC curves for the cut-off value of 0.08 (Figure 2) showed excellent results with an AUC of 0.952 (95% CI, 0.927 to 0.978) and 0.934 (95% CI, 0.902 to 0.966) for males and females respectively. A further indication of an excellent test result can be observed as the ROC curves demonstrate an initial rise vertically from the lower left corner and then moves horizontally along the upper line[22].

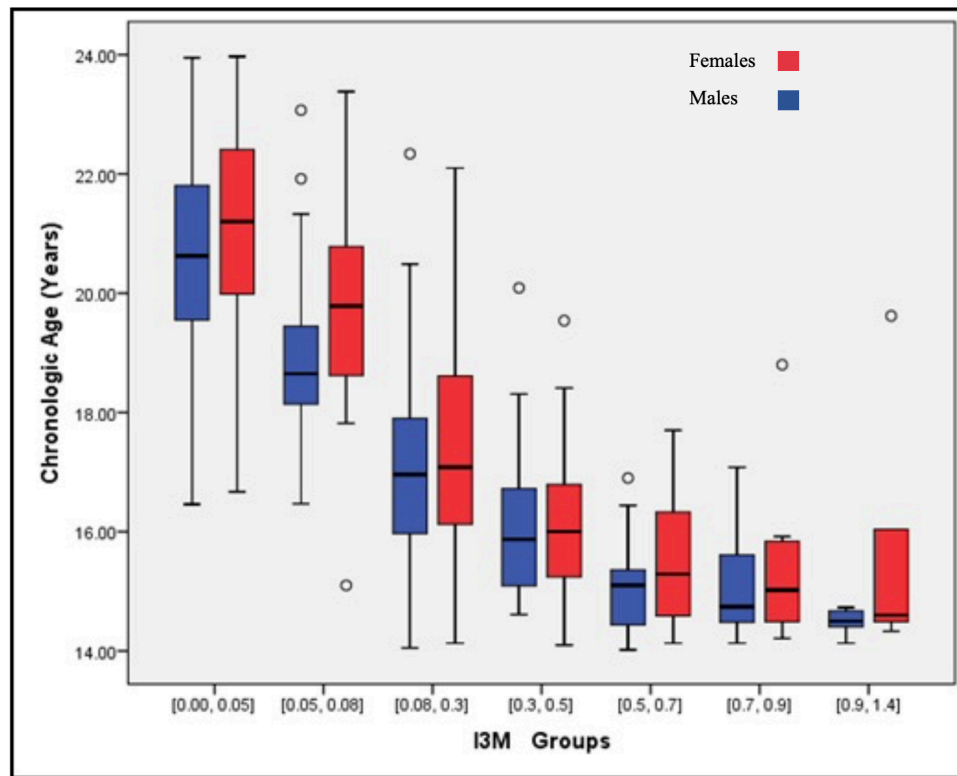**Figure 1.** Boxplot Graph showing relation of Chronologic Age & I3M Classes.

Table 2. Summary of descriptive statistics of chronological age according to third molar maturity index (I3M)

I3M	Males								Females							P*
	N	Mean	SD	Min	Q1	Median	Q3	Max	N	Mean	SD	Min	Q1	Median	Q3	Max	
[0.00,0.05]	117	20.54	1.60	16.46	19.52	20.61	21.81	23.95	123	21.10	1.66	16.67	19.94	21.20	22.45	23.97	0.349
[0.05,0.08]	27	18.84	1.57	16.47	18.05	18.65	19.53	23.07	30	19.88	1.68	15.10	18.61	19.79	20.81	23.38	0.259
[0.08,0.3]	58	17.00	1.58	14.05	15.91	16.96	17.91	22.34	67	17.32	1.87	14.13	16.12	17.08	18.65	22.10	0.795
[0.3,0.5]	21	16.08	1.36	14.61	15.03	15.87	16.78	20.09	21	16.12	1.31	14.10	15.12	16.00	16.80	19.54	0.302
[0.5,0.7]	17	15.12	0.83	14.02	14.37	15.10	15.67	16.90	17	15.58	1.21	14.13	14.51	15.29	16.59	17.70	0.642
[0.7,0.9]	22	15.03	0.76	14.13	14.48	14.74	15.61	17.08	9	15.41	1.42	14.21	14.42	15.02	15.88	18.80	0.223
[0.9,1.4]	7	14.50	0.21	14.13	14.35	14.50	14.69	14.73	6	15.61	2.06	14.33	14.45	14.60	16.94	19.62	0.317

Legend : * Mann Whitney U Test, Number of individuals (N), Mean, standard deviation (SD), minimum value (Min), 1st quartile (Q1), median, 3rd quartile (Q3) and maximum value (Max) of age distribution for each I3M class, for females and males.

Figure 2. Receiver Operator Characteristic Curve for I3M for males & females
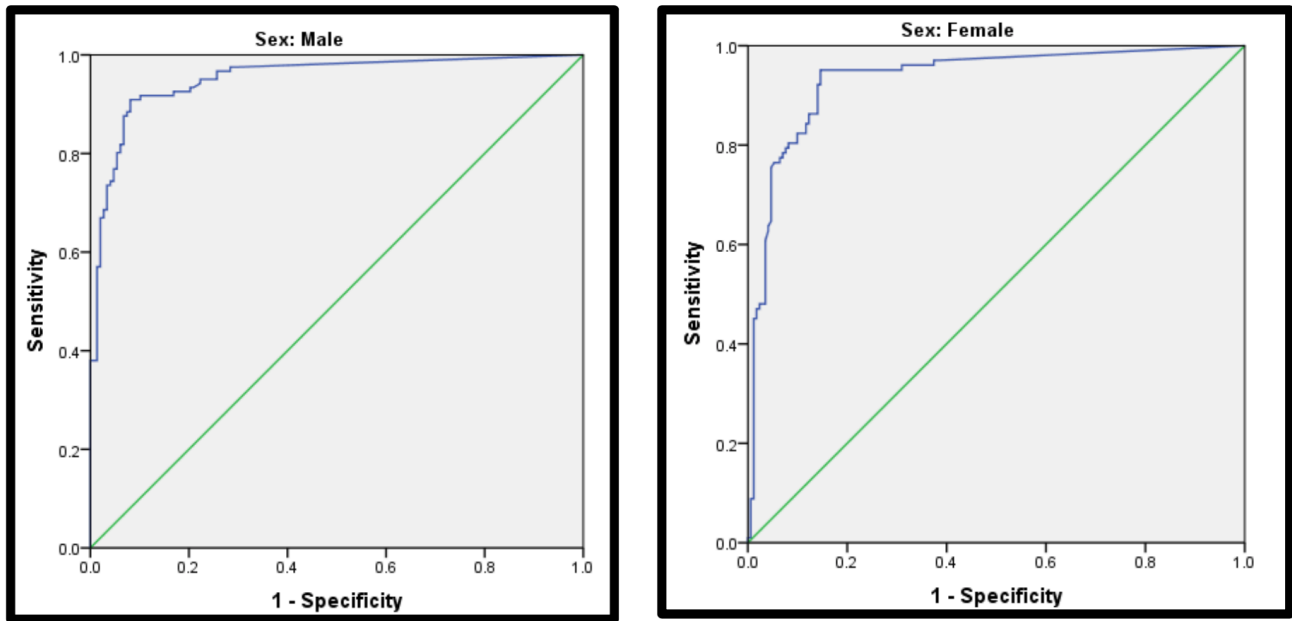

Table 3. Contingency table describing discrimination performance of the test for different cut-off values of third molar maturity index (I3M) in males and females.

	I3M (Males)				I3M (Females)		
	Age				**Age**		
Test	**≥18**	**<18**	**Total**	**Test**	**≥18**	**<18**	**Total**
I3M < 0.02	106	4	110	**I3M < 0.02**	107	3	110
I3M ≥0.02	42	117	159	**I3M ≥0.02**	64	99	163
I3M < 0.04	110	4	114	**I3M < 0.04**	112	4	116
I3M ≥ 0.04	38	117	155	**I3M ≥ 0.04**	59	98	157
I3M < 0.06	118	8	126	**I3M < 0.06**	134	5	139
I3M ≥ 0.06	30	113	143	**I3M ≥ 0.06**	37	97	134
I3M < 0.08	133	11	144	**I3M < 0.08**	146	7	153
I3M ≥ 0.08	15	110	125	**I3M ≥ 0.08**	25	95	120
I3M < 0.1	136	14	150	**I3M < 0.1**	147	13	160
I3M ≥ 0.1	12	107	119	**I3M ≥ 0.1**	24	89	113
I3M < 0.12	138	16	154	**I3M < 0.12**	150	15	165
I3M ≥ 0.12	10	105	115	**I3M ≥ 0.12**	21	87	108
I3M < 0.14	138	17	155	**I3M < 0.14**	150	16	166
I3M ≥ 0.14	10	104	114	**I3M ≥ 0.14**	21	86	107
Total	**148**	**121**	**269**	**Total**	**171**	**102**	**273**

Table 3 shows a consolidated 2x2 contingency table for various age groups. Tables 4 and 5 are comparative tables showing derived values for various I3M cut-off values for males and females respectively.

In males, as observed in Table 4, for the cut-off value 0.08 the AC, Sp, J-Index and Bayes PTP were 0.903, 0.909, 0.808 and 0.949 respectively. I3M cut-off of 0.06 performs better when Sp and Bayes PTP are considered but the AC and J index are significantly lower at 0.859 and 0.731 respectively. The cut-off of 0.1, 0.12 and 0.14 shows marginally better performance when Ac and Se are considered but under-performs in other parameters.

In females, as observed from the findings of Table 5, for the I3M cut-off of 0.08, the AC, Sp, J Index and Bayes PTP was 0.883, 0.931, 0.785, 0.955

respectively. The I3M ratio of 0.06 shows better Sp of 0.951 but also has a lower Ac and J Index of 0.846 and 0.735 respectively. The I3M cut-off of 0.08 out-performs the cut-off of 0.1 in most other parameters.

From a practical standpoint, medico-legal tests used to estimate legal adulthood, must give appropriate weighting to the cut-off value with higher specificity while maintaining adequate results of other parameters. It is important to ensure that the test shows a low proportion of false positives (minors identified as adults). The gradual decrease in the PPV, LR+ and LR- values and an increase in the NPV values is an expected result as the cut-off value increases. Therefore, the I3M cut-off of 0.08 shows the best accuracy and reliability for both males and females.

Table 4. Comparative chart showing values derived from contingency table for various I3M cut-off values to determine legal age in Indian Goan population

	I3M Male						
	0.02	**0.04**	**0.06**	**0.08**	**0.1**	**0.12**	**0.14**
AC	0.829	0.844	0.859	0.903	0.903	0.903	0.900
Sensitivity	0.716	0.743	0.797	0.899	0.919	0.932	0.932
Specificity	0.967	0.967	0.934	0.909	0.884	0.868	0.860
J-index	0.683	0.710	0.731	0.808	0.803	0.800	0.792
PPV	0.964	0.965	0.937	0.924	0.907	0.896	0.890
NPV	0.736	0.755	0.790	0.880	0.899	0.913	0.912
LR+	21.666	22.483	12.059	9.885	7.942	7.052	6.637
LR-	0.293	0.266	0.217	0.111	0.092	0.078	0.079
Bayes PTP	0.976	0.977	0.958	0.949	0.938	0.930	0.926

Legend: AC accurate classification, Se sensitivity, Sp specificity, J-index Youden index, PPV positive predictive value, NPV negative predictive value, LR+ positive likelihood ratio, LR-, negative likelihood ratio, Bayes PTP Bayes post-test probability

Table 5. Comparative chart showing values derived from contingency table for various I3M cut-off values to determine legal age in Indian Goan population.

	I3M Female						
	0.02	**0.04**	**0.06**	**0.08**	**0.1**	**0.12**	**0.14**
AC	0.755	0.769	0.846	0.883	0.864	0.868	0.864
Se	0.626	0.655	0.784	0.854	0.860	0.877	0.877
Sp	0.971	0.961	0.951	0.931	0.873	0.853	0.843
J-index	0.596	0.616	0.735	0.785	0.732	0.730	0.720
PPV	0.973	0.966	0.964	0.954	0.919	0.909	0.904
NPV	0.607	0.624	0.724	0.792	0.788	0.806	0.804
LR+	21.275	16.702	15.986	12.441	6.745	5.965	5.592
LR-	0.386	0.359	0.228	0.157	0.161	0.144	0.146
Bayes PTP	0.973	0.966	0.965	0.955	0.920	0.911	0.906

Legend: AC accurate classification, Se sensitivity, Sp specificity, J-index Youden index, PPV positive predictive value, NPV negative predictive value, LR+ positive likelihood ratio, LR-, negative likelihood ratio, Bayes PTP Bayes post-test probability.

DISCUSSION

In India the Juvenile Justice Act exempts minors from criminal liability and they are subject to special criminal standards[23]. Many perpetrators claim to be juveniles in the hope of a lenient trial and verdict when arrested and presented before the court. The authorities and courts have often relied upon forensic odontologists for age estimation to offer an evidence-based methodology for this issue. In such cases the fundamental question that needs to be answered, is the subject a major or a minor rather than the exact age.

Age estimation of non-adults can be performed by using skeletal indicators or dental age estimation. There are reports that question the accuracy of skeletal indicators due to variations in bone development, which can be influenced by nutritional and environmental factors[24]. Furthermore, ossification of hand and wrist bones is completed by 18 years and thus cannot be used to evaluate legal age[25]. Tooth development on the other hand, is controlled more by genetics rather than by environmental and nutritional factors[24]. Dental development is independent of the exogenous factors such as nutrition[26], disease[26-28], mental stress[26,27], pathology [29] and environmental factors[30]. Among subjects over the age of 14 years, radiographic evaluation of mandibular third molars is often relied upon as it is the only tooth continuing to develop at this age[31]. Cameriere's study on the third molar maturity index provides us with an objective and reliable technique to determine legal age. It was found that geographical differences between third molar development are of negligible clinical influence and thus can be considered and applied beyond geographic boundaries[29,32].

The studies on I3M have been carried out on a wide demography which includes North America[12], South America[17], Europe[7,9,13,16], Africa[4,11,15], Asia[25] and Australia[14]. Balla S.B. et al carried out the first Asian study on South Indian population[25]. Our study was the second study carried out in an Indian population and the first study in a Goan population.

Our sample included Indian Goans ranging from 14 to 24 years of age. The first case of apical closure was noted at 17 years in both males and females which is consistent with findings of other studies[15,16]. Hence, it could be stated that the minimum age for apical closure is 17 years for both sexes.

Many studies show evidence that there is an overall lower degree of third molar development in females compared to males[15,16]. The same can be observed in the present sample (Table 1) which could indicate a possible male precedence in the development of third molars. However, there was no statistical difference noted on comparison of mean ages within I3M classes for both sexes.

For the cut-off value of 0.08, our study noted an AUC of 0.95 and 0.93 for males and females respectively which is comparable with the results obtained by other studies[15,17]. Comparatively, 90% males were accurately classified as compared to 88% for the females. The Se and Sp for males (0.89, 0.90) and females (0.85, 0.93) were comparable with other studies[7-10,12,15-17].

The PPV, NPV and LRs are useful in forensic odontology to analyse the probability of an individual being at least 18 years of age. The lesser false positives (higher specificity) signify a higher PPV in the observed population and lesser false negatives (higher sensitivity) will have a higher NPV in the observed population. We noted PPV values of 0.92 and 0.95 for males and females respectively.

The LR values combine sensitivity and specificity into a single figure that indicated by how much the test result will reduce the uncertainty of a given diagnosis. The LRs allow the researcher to study the ability of a test to alter a pre-test probability of being a major into a post-test probability. A LR+ > 10 and a LR- < 0.1 are considered to exert highly significant changes in probability.[16] If the LR value is close to 1 it indicates a worthless test. Studies by Cavric J. et al[15], Zelic K et al[16] and Quispe- Lizarbe Roselhy, J. et al[17] recorded high LR+ values ranging from 13 - 37. The lowest LR+ was noted by Franklin D et al[14] at 6.04. In the present study the LR+ was 9.88 in males and 12.4 in females. This would indicate that the likelihood of being at least 18 years, if the mandibular third molar had achieved apical closure was at least 9 and 12 times more likely in males and females respectively. The LR- was recorded at 0.11 and 0.15 for males and females respectively. A LR- of less than 0.1 was noted in some studies[13,16,17]. A majority of the studies noted LR- between 0.1 – 0.15 [8,14,15].

The Bayes PTP indicates the probability of an individual who was classified as a major to

factually being a major. This calculation factors in the proportion of individuals who are above 18 years in the representative demography. Bayes PTP for our study was 0.95 for both males and females.

The J-Index captures the performance of a dichotomous diagnostic test. It is a function of sensitivity and specificity. It denotes the maximum distance of the ROC Curve from the diagonal line. The value ranges from 0 to 1. A value of 1 indicates a perfect test whereas, 0 indicates a worthless test. Cavric J et al[15] noted a value of 0.82 and 0.84 for males and females respectively. The present study showed comparable results 0.8 and 0.78 for males and females respectively.

On comparison of various cut-off values (Tables 4 and 5), I3M cut-off value of 0.08 showed the best performance I3M with respect to AC, J index, Sp and Bayes PTP. The cut-off value of 0.1 showed marginally higher Se for both sexes. This increase in Se does not justify selecting 0.1 as a cut-off value over 0.08, as the Sp and post-test probability is higher for the cut-off value of 0.08. A similar inference was made by Cavric J. et al[15].

In conclusion, the third molar maturity index as described by Cameriere et al[5] offers a reliable and objective method for estimation of legal adulthood. According to Corradi et al[33] 51% of correct classification may be sufficient for civil cases with "more probable than not" evidence while very high levels, at least 90 %, was needed for criminal cases which require "beyond all reasonable doubt" evidence. Hence, the I3M cut-off of 0.08 can be utilised reliably for forensic application in criminal and judicial courts.

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