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**Forensic Dental Age Estimation of Sub-Adult Individuals Using Nolla's
Radiographic Method: A Systematic Review and Meta-Analysis**

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Abstract. Nolla's radiographic method of dental age estimation for sub-adult individuals has been commonly used for forensic purposes. To identify the accuracy and credibility of this method, a review has been conducted for estimating the variations/discrepancies between actual chronological and estimated ages of sub-adults individuals calculated by applying Nolla's radiographic method in different population groups. The articles using Nolla's method of age estimation and those published between 2006 and 2016 were used after relevant searches in PubMed, EMBASE, SCOPUS, COCHRANE and Web of Science databases. Studies conducted with dental radiographs of healthy subjects between 3 and 18 years of age and those reporting mean differences between the estimated dental age (DA) and chronological age (CA) as a measure of variation were included in the review for meta-analysis. A total of 5813 subjects (2839 boys and 2974 girls) between the age of 3 and 18 years were examined in the study, which were chosen from a total of 87 peer-reviewed articles. Quantitative (10 studies) and qualitative (12 studies) analyses of the results revealed that dental age of most sub-adults was found under-estimated using this method based developmental stages of teeth. The underestimation varied from +0.26 to +1 years for males, and +0.051 to +1.15 for females; with an average mean of 0.35 years and 0.20 years for males and females, respectively. Thus, it can be concluded that Nolla's method of dental age estimation underestimates the age of an individual in almost all the studies reviewed here. It should be carefully applied for forensic cases requiring legal scrutiny by courts of law.

Keywords: Forensic anthropology; Age estimation; Nolla's radiographic method; Dental developmental stage; Meta-analysis.

1. Introduction

Dental remains offer the most rapid, reliable and useful method of forensic identification to scientific community and investigating agencies to identify victims of mass disasters, criminal activity like terrorism or natural catastrophe¹. Age assessment of the mutilated/decomposed corpse or skeletal remains is one of the most challenging tasks encountered by forensic experts engaged in establishing biological profile of such human remains. Dental age estimation methods are of particular value in forensic anthropological analyses as teeth are found highly resistant to mechanical, physical, chemical or biological degradations or taphonomic destructions. Teeth have the longest post-mortem longevity among all human skeletal remains. For studying various age-dependent changes in teeth; a number of morpho-histological, molecular or radiographic techniques have been commonly employed. However, histological and molecular examinations of teeth give comparatively more precise and credible results for adult teeth, though radiological techniques are commonly used for age assessment of developing teeth of adolescents^{2,3}. The tooth with its developmental phases impart noninvasive, nondestructive attribute to assess the person's age. A number of systems³ have been used to estimate the dental age as stated by the extent of the developmental stages reported in radiographic examinations in permanent teeth. Among all methods of age estimation for sub-adult teeth, Nolla's radiographic method⁴ based on dental development stages is generally used for both teaching and research assignments in diverse fields of forensic sciences. This method assesses the stages of mineralization of permanent dentition on X-ray radiographs to estimate age threshold which is divided into ten maturation stages of all teeth of both upper and lower dental arch⁵.

Over the past few decades, numerous studies have been published using Nolla's method⁴ for age assessment of sub-adult dentition⁶⁻¹⁷. So far, no meta-analysis of different studies has been published to estimate the reliability and accuracy of Nolla's method in evaluating age of sub adult individuals. Present study authors carried out a systematic review and meta-analysis of findings presented by various

researchers by comparing the level of their estimations (underestimation or overestimation) with the aim of answering a question: what is the accuracy and credibility of Nolla's method for forensic age estimations of individuals of diverse population groups?

2. Materials and methods

2.1 Search design

A computerized search of articles, which employed Nolla's method of dental age assessment and those published in English language journals/books between years 2006 to march 2016, was performed. The screening of articles was independently done by the authors for inclusion in this study. The search includes PubMed, EMBASE, SCOPUS, COCHRANE and Web of Science databases and was filtered by applying all the related Medical Subject Heading (MeSH) terms and text words like Nolla method; dental age; Nolla's developmental stages; age determination/estimation; age determination by teeth; panoramic; pantomography; panoramic radiography; radiograph; radiology; application etc. Further, relevant study articles were searched and retrieved from reference lists also to accommodate additional potential sources.

2.2 Inclusion criteria

Only the relevant research articles using Nolla's method for age determination were included. Studies conducted with dental radiographs of healthy subjects between 3 and 18 years of age and those reporting mean differences between the estimated dental age (DA) and chronological age (CA) as a measure of variation were included in review for meta-analysis. Methods of the selection process, literature search and exclusion criteria of studies in present systematic review have been displayed in flow diagram (Fig.1).

2.3 Exclusion criteria

Research studies having subjects with any developmental anomalies or chronic illness, lacking any information on health conditions; using subjects taking nutritional supplementations or hormonal replacement therapies, without mention of intra- or inter-observer variability, having results presented in percentages or in median and those

conducted in other languages or prior to year 2006 were not included in this meta-analysis in an attempt to avoid biasness in the results.

2.4 Data sources

PubMed, EMBASE, SCOPUS, COCHRANE and Web of Science databases were searched for the relevant studies via Google search engine from Windows 10 explorer. Data was collected from the studies published between 2006 and 2016 (up to March). The time-interval was arbitrarily selected to include sufficient number of studies in this systematic review. Significant numbers of articles were examined with similar search conditions from the journals like Journal of Forensic Odontostomatology, Forensic Science International, International Journal of Pediatric Dentistry and Journal of Forensic Sciences.

2.5 Characteristics of included studies

A total of 87 articles from peer-reviewed journals were selected as meaningful to justify the aims and objectives of present review. Out of these, 71 articles were rejected as 'irrelevant studies' (after getting inspected the article titles and abstracts) in order to meet the exclusion criteria followed in this meta-analysis. The excluded studies had either used other methods of age assessment, reported results in percentages or in median, with no reference of male and female subjects or the subjects were taking some dietary supplements. Disagreement was resolved by discussion among the review authors during the selection of articles. Finally, 12 relevant studies were retained for the qualitative synthesis and 10 for quantitative analysis or meta-analysis⁶⁻¹⁷. The process of study, screening and literature search are depicted in Figure 1.

A total of 5813 subjects (2839 male and 2974 female) from 12 qualitative studies, between 3 and 18 years of age, were included in present systematic review. Of the 12 included studies employing Nolla's method; 5 studies were retrospective, 3 were retrospective cross-sectional, 1 cross-sectional and 3 unknown (without mention of retrospective or cross-sectional) studies. All the studies expressed the mean age difference (difference between the CA and estimated DA or vice versa). The mean age differences, if it was given in months, were then converted into years in a decimal value^{7,13}. For the studies not specifying age estimation differences, the mean

differences were acquired by thoroughly investigating values from the table^{7,13,15,16}. The characteristic description of all included studies has been outlined in Table 1.

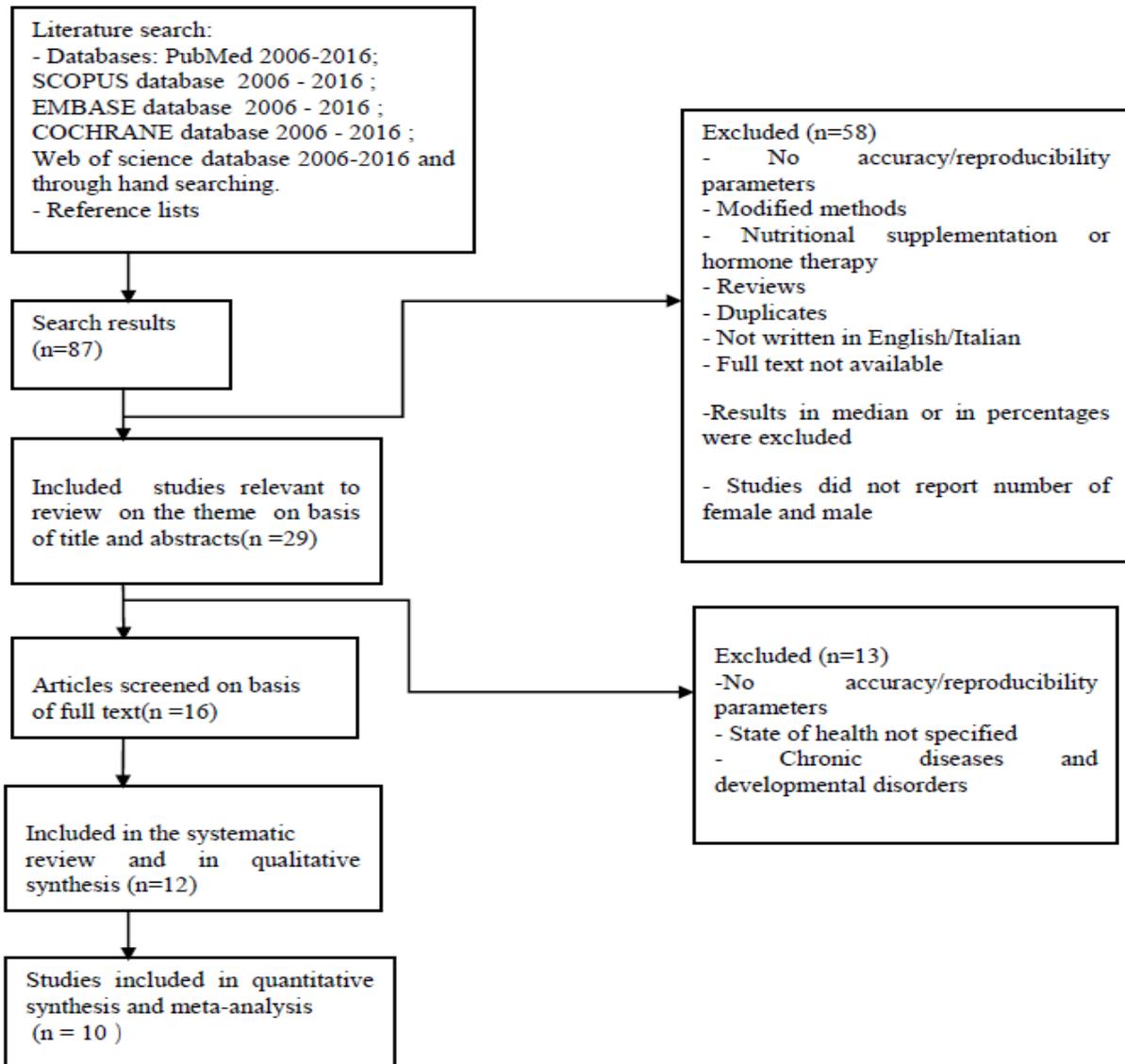


Figure 1. Flow diagram of the selection process of the included studies and the specific reasons for exclusion from the present meta-analysis.

Table 1. Age estimation results among different population groups using the Nolla's method.^a Converted value; ^b Table value calculation.

Country	Author	Place	Research type	Ethnicity	Sample size			Age (years)	Age estimation difference(CA-DA) years	
					Total	Females	Males		Males	Females
Turkey	Bilge Nur et al. ¹⁷	Trabzon	Retrospective study	Not stated	673	331	342	5 to 16	-0.5	-0.57
India	Rai and Anand ⁹	Haryana	Retrospective cross-sectional study	Asian, Indian	75	35	40	5 to 14	0.29	0.27
India	Thomas et al. ¹⁶	Mangalore	Not stated	Asian, Indian	25	10	15	3 to 16	0.02 ^b	0.051 ^b
UK	Maber et al. ⁸	London	Retrospective cross-sectional study	White, British and Asian, Bangladeshi	946	455	491	3 to 16.99	-0.87	-1.18
Turkey	Kirzioglu and Ceyhan ⁶	Isparta	Retrospective study	Not stated	425	213	212	7 to 13	-0.53	-0.57
India	Rai ¹³	New Delhi	Not stated	Asian, Indian	413	206	207	6 to 16	0.276 ^a b	-0.144 ^{ab}
Malaysia	Kumaresan et al. ¹⁴	Semeling	Retrospective cross-sectional study	Asian, other	426	247	179	5 to 16	0.5	0.57
Brazil	Kurita et al. ⁷	Fortaleza	Not stated	Not stated	360	180	180	7 to 15	0.883 ^a b	1.15 ^{ab}
India	Mohammed et al. ¹¹	Visakhapatnam, Andhra Pradesh	Cross-sectional	Asian, Indian	660	330	330	6 to 16	0.32	0.62
Turkey	Altunsoy et al. ¹⁵	Izmir	Retrospective study	Not stated	688	331	357	7 to 17	0.52 ^b	0.69 ^b
Turkey	Miloglu et al. ¹⁰	Ataturk	Retrospective study	Caucasian Turkish	719	409	310	7 to 18	-0.2	-0.5
Pakistan	Khoja et al. ¹²	Karachi	Retrospective study	Asian, Pakistani	403	227	176	8 to 16.9	1	-0.21
<i>Total</i>	-	-	-	-	5813	2974	2839	-	0.142	0.014

3. Results

The accuracy of Nolla's method examined in present meta-analysis has been explained gender-wise as given below:

3.1 Comparisons between chronological age (CA) and dental age (DA) in males

3.1.1 Qualitative analysis

Underestimation of age was found generally in all the examined studies, except for a few. Conversely, overestimation of the age was reported only in Turkey and UK studies reported by Nur et al.¹⁷, Zuhail et al.⁶, Miloglu et al.¹⁰ and Maber et al.⁸. While evaluating the effects of all referenced studies, the variations in the estimated age ranged from + 1 to years to -0.87 years (Fig. 2). Minimum differences were reported for Turkish and Indian studies by the Miloglu et al.¹⁰ and Thomas et al.¹⁶; with a difference in mean of -0.2 and +0.02 years, respectively. Maximum difference of +1 year/s was reported for Khoja et al.¹² study for Pakistan individuals. The mean differences for all studies was estimated as +0.14 years for male.

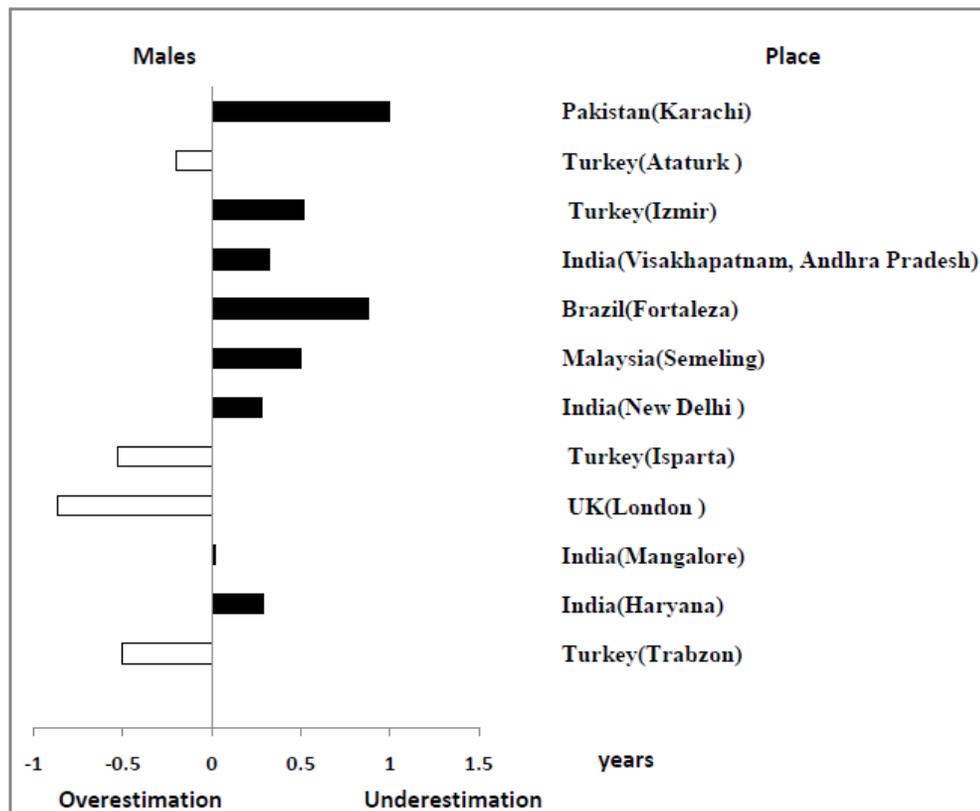


Figure 1. Variations in the age (CA-DA) for males among different populations using the Nolla's method.

3.1.2 Quantitative analysis

An outline of the meta-analysis results for males regarding interrelationship between estimated dental age (DA) using Nolla's method and the reported chronological age (CA) has been shown in Figure 2. Considering heterogeneous nature of different population groups included in this systematic review, the 'random effects model' of meta-analysis was applied. As presented in Figure 2, statistically significant overestimation was reported for Malaysian and Indian subjects studied by Kumaresan et al.¹⁴ and Mohammed et al.¹¹, respectively; and other studies reported underestimation of age. The age underestimation was found highly significant for Pakistani individuals studied by Khoja et al.¹² (WMD= +1, (0.43, 1.57) 95% CI). Overall, meta-analysis showed a substantial difference between the chronological age (CA) and the dental age (DA) (WMD = 0.35, 95%CI = 0.07, 0.63, P = 0.0005) of individuals of almost all studies considered in this meta-analysis and explained in Figure 2.

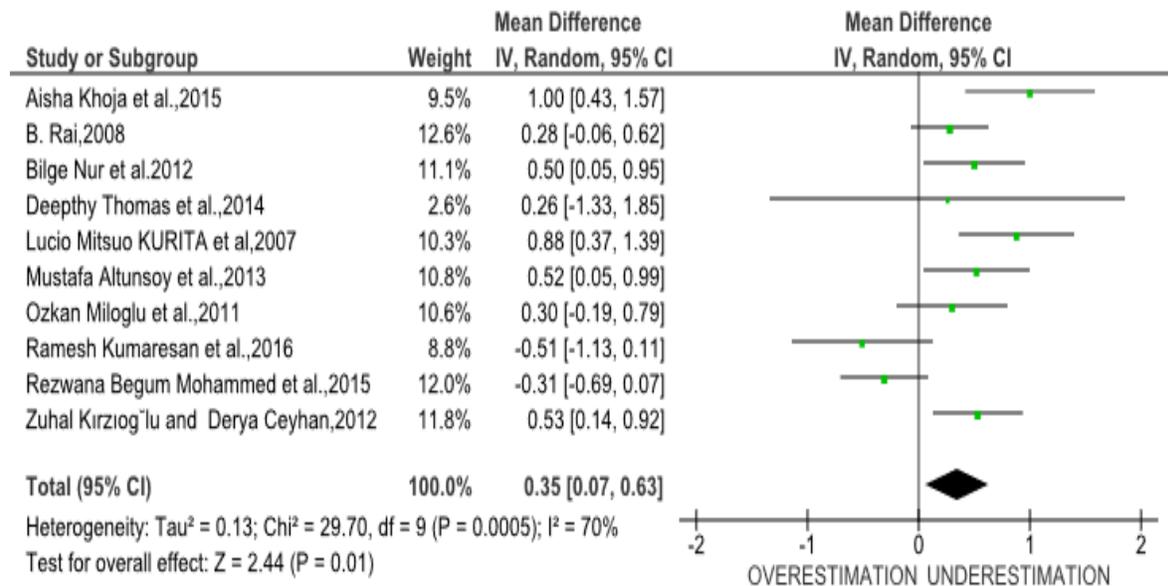


Figure 2. Forest plot of mean differences in the age (CA-DA) among males derived from Nolla's method.

3.2 Comparisons between chronological age (CA) and dental age (DA) in females

3.2.1 Qualitative analysis

Almost half of the studies showed underestimation of age in females (Fig. 3). The mean difference of all 12 studies (involving females) was found as 0.014 years. While comparing the age estimation results for global population, the variations in the estimated age from Nolla's method ranged from -1.18 years to +1.15 years when compared with the chronological age (Fig. 3). Minimum (0.051 years) and maximum (-1.18 years) differences in ages were reported for Mangalore (India) subjects studied by Thomas et al.¹⁶ and British individuals studied by Maber et al.⁸ respectively.

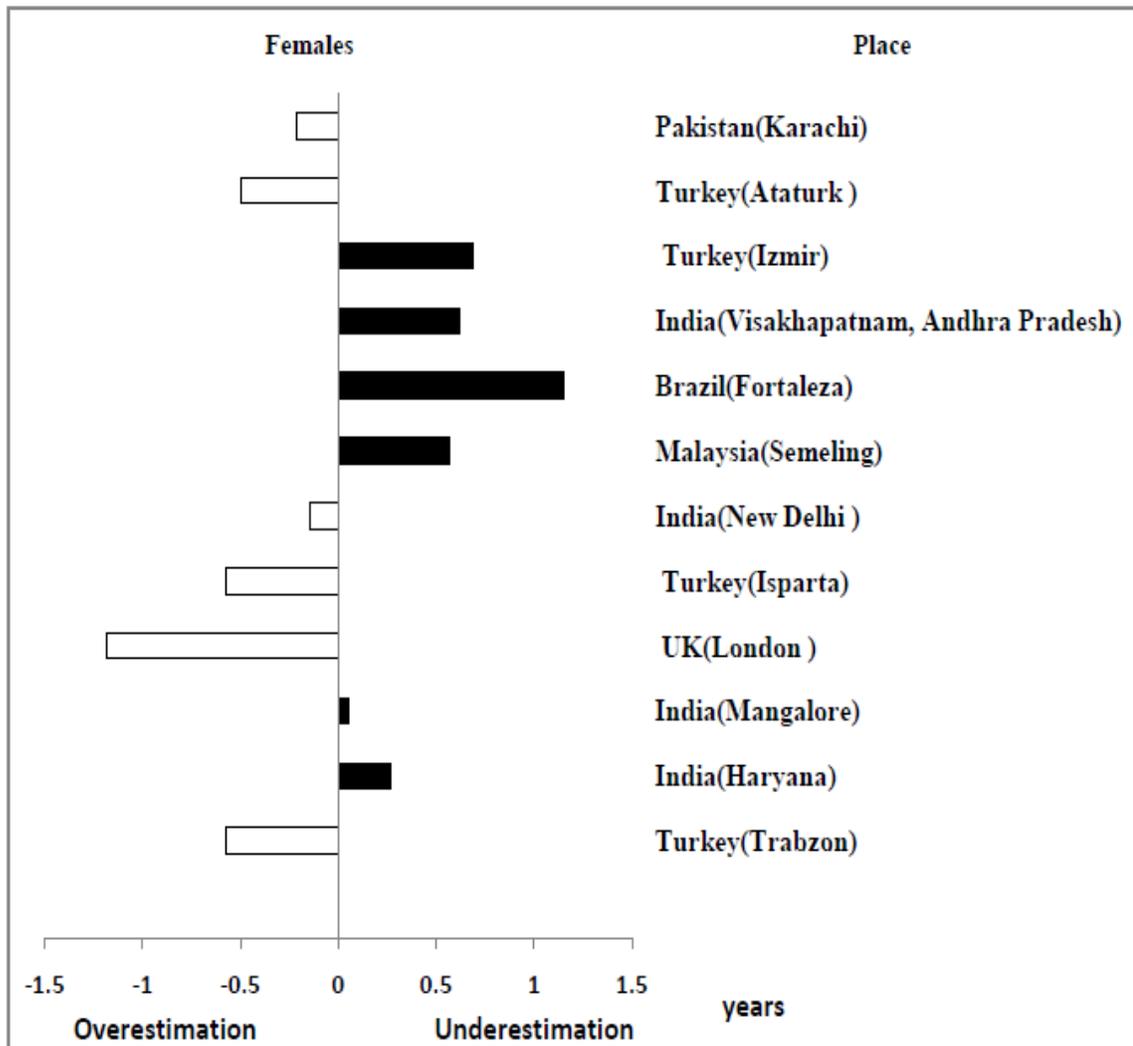


Figure 4. Variations in the age (CA-DA) for females among different populations using Nolla's method.

3.2.2 Quantitative analysis

Figure 4 displays the quantitative comparison of the chronological age (CA) and estimated dental age (DA) of females from Nolla's method. Considering heterogeneous nature of different population groups whose females were included in this systematic review, the 'random effects model' of meta-analysis was applied. The overall mean difference between chronological age (CA) and estimated dental age (DA) was found as 0.20 years [-0.17, 0.58] 95% CI, $P < 0.00001$). The highest overestimation and underestimation were reported for Indian (WMD= -0.63[-1.00,-0.26] 95% CI) and Brazilian (WMD= +1.15[+0.64, 1.66] 95%CI) subjects studied by Mohammed et al.¹¹ and Kurita et al.⁷, respectively.

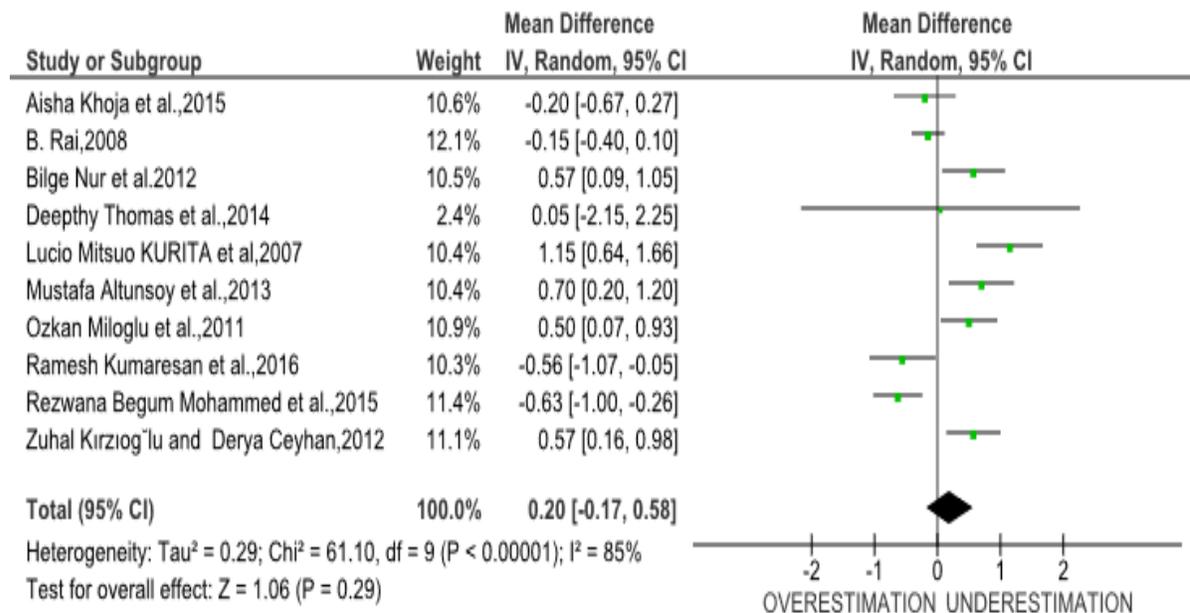


Figure 4. Forest plot of mean differences in the age (CA-DA) among females derived from Nolla's method.

3.3 Estimation of Publication Bias

Publication bias is a menace in qualitative and quantitative studies, review and meta-analysis. Funnel plots were applied to evaluate the potential publication bias, if any. Asymmetry in funnel plots may indicate publication bias in meta-analysis. When no publication bias is present in the plot should show a funnel-like shape, with estimates spanning down from the larger trials symmetrically in both directions with increasing

variability. A plot of effect estimate against its standard error for a single outcome. The shapes of the plots did not represent significant asymmetry (Fig. 5).

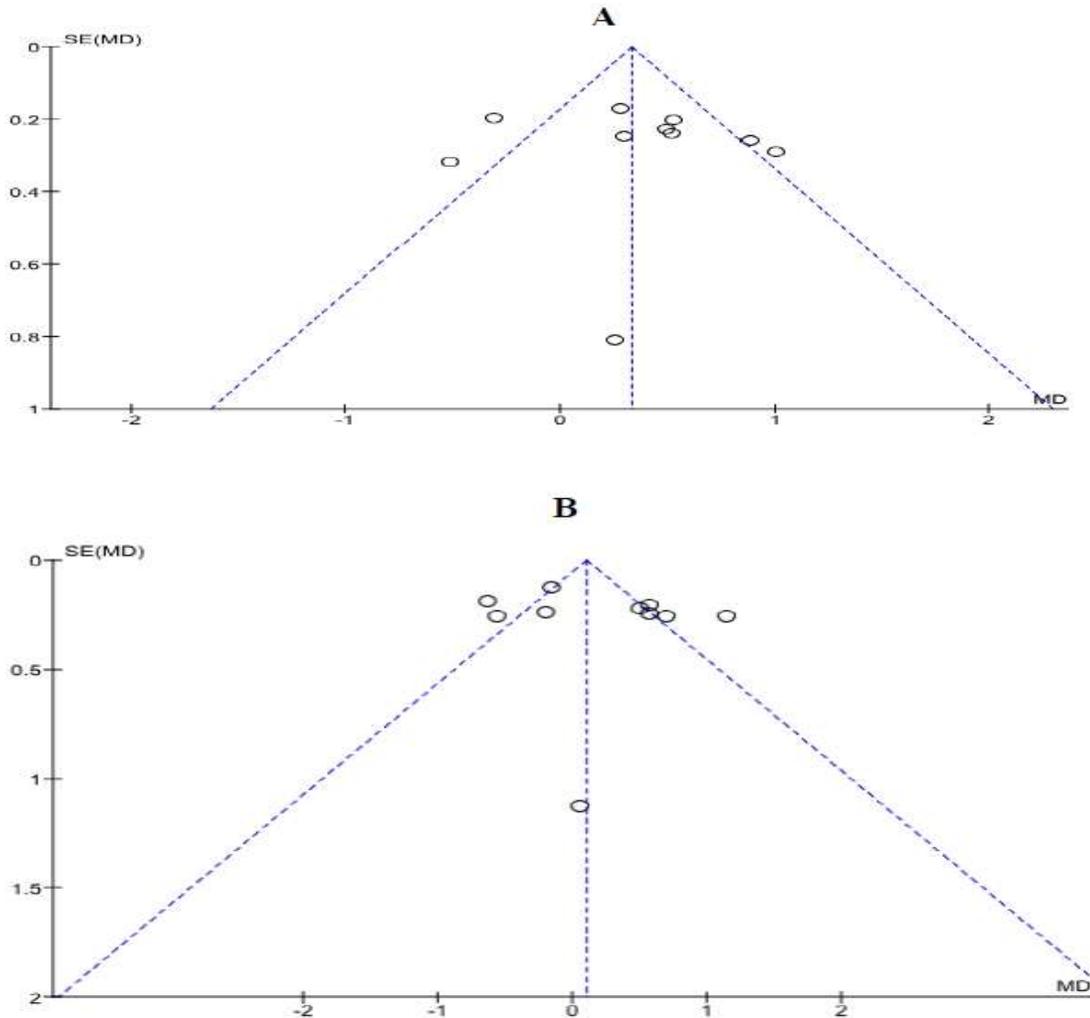


Figure 5. Begg's funnel plot of the meta-analysis of the difference between dental ages using Nolla's method and chronological age among Males (A) and Females (B). Each point represents a separate study for the indicated association

4. Discussion

Dental development and its emergence patterns have long been accepted as a useful parameter for age estimating. Nolla's technique⁴ of dental age assessment calculates the mineralization status of permanent teeth through standard ten stages and, it is a universally accepted system for calculating dental ages of wider ranges. It utilizes developmental stages of teeth for age assessment, which was initially applied to a

limited sample of 25 boys and 25 girls from the files of the child development laboratories of the University of Michigan School in 1960. In recent past, the suitability of this method has been broadly examined in diverse population groups and, uncertainties have been raised about reliability of this method. Therefore, present study authors aimed to carry out a meta-analysis of various studies published (during the decade of 2006-2016) which used Nolla's method for age estimation of sub-adult individuals. Studies that used the Nolla's method and reported the results in differences in mean (CA-DA or vice versa) were included in order to examine the total difference in dental age among the diverse population groups.

The main aim of the current review was to conclude the mean variations between actual chronological and estimated ages of sub-adults calculated by applying Nolla's method. A total of 5813 subjects (2839 male and 2974 female) from 12 published studies⁶⁻¹⁷ were included in this systematic review. A quantitative meta-analysis was also conducted based on 10 studies^{6-7, 10-17} for expressing the mean estimates (overestimation or underestimation) and the standard deviation of their estimates.

Our findings illustrated that dental age of children from Nolla's method were under-estimated lower than their chronological age. The underestimation of dental age differs from +0.26 to +1 years for males, and +0.051 to +1.15 years for females, with an average mean of 0.35 and 0.20 years for males and females, respectively. The highest underestimation of dental age were reported for the Pakistani individuals studied (WMD= +1 year, 95% CI (0.43, 1.57)) by Khoja et al.¹² and Brazilian subjects (WMD= +1.15[+0.64, 1.66] 95%CI) studied by Kurita et al.⁷, for males and females, respectively. Similarly, Jayaraman et al.¹⁸ found that Dermirjian dataset overestimated the age of males by 0.60 years (-0.23 years to +3.04 years) and females by 0.65 years (-0.1 years to +2.82 years), thus overestimating the age of both the sexes by six months on an average. It may be inferred from this analysis that dental variability in maturity processes existing among distant population groups may be the reason for differences in age estimation thresholds. It has also been agreed upon that the reported variations might have been further affected by the methodology, sample size and its distribution, examiner expertise, inter-individual variability or the statistical testing carried out.

Thus, our estimation of the accuracy of the Nolla's method based on the findings of the systematic literature review has some restriction that also include the exclusion of articles on the basis that they showed the results in median and in percentages, exclusion of articles with no information on health status and articles which were published other than English language. The present meta-analysis results warrant for further exploratory studies to corroborate or negate the quantitative and qualitative observations of this meta-analysis. No other meta-analysis based on dental age estimations was available to the authors from the accessible literature. Articles published between 2006 and 2016 were arbitrarily included in the present study because those were recent studies which alleged to give precise estimates than those published before 2006. It is worthy to note that a secular trend in the dental maturity has been reported between subjects from two different decades¹⁹.

Besides some constraints, current meta-analysis possesses some significant findings. According to the literature accessible to the authors, this is the first meta-analysis on age estimation of sub-adult individuals using Nolla's method. Result clearly indicates Nolla's method underestimates dental age in nearly every population study for both the sexes. Consequently, it becomes necessary to take into account further research on different geographic region of population to establish different estimation calculations so be able to obtain most accurate and precise dental age estimates.

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