EVALUATION OF DENTAL ERUPTION AND SECONDARY SEXUAL CHARACTERISTICS IN HEALTHY GIRLS WITHIN THE 14-16 YEARS AGE GROUP: A COMPREHENSIVE STUDY

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Abstract

For several decades, dental radiographs have been utilized to determine chronological age, serving purposes such as forensic identification, monitoring migration patterns, and evaluating dental development, among other applications. Tooth eruption, a developmental morphology criterion, can be assessed through either clinical examination or the evaluation of dental radiographs for the determination of dental age. A research investigation was undertaken to conduct synchronized assessments of dental eruption and secondary sexual characteristics. One hundred healthy schoolgoing adolescent females, aged 14-16 years, were examined to assess the status of dental eruption, including the space behind the second molar tooth, along with observations of secondary sexual characteristics. All the subjects were given questionnaire to collect information regarding their dietary habit, socio-economic status and exercise habits. Observations regarding the degree of epiphyseal union, presence or absence of space behind the second molar tooth, stage of second molar tooth eruption and stage of development of pubertal changes were recorded for each subject, statistically analysed. Eruption of the second molar tooth was complete in 96% of girls aged 14-15 and, in all girls, aged 15-16. The development of the space behind the second molar tooth is significant in the lower jaw by the age of 15-16 in females. Pubic hair development mostly ranged from Stage 3 to Stage 4 in females aged 14-16, while breast development varied from Stage 2-3 in 14-15-year-olds to Stage 3-4 in 15-16-year-olds. The mean age of menarche was 12.34 years.

Keywords: Tooth Eruption, Dental Age, Pubic Hair, Menarche, Breast Development.

INTRODUCTION

Establishing an individual's identity often involves assessing their age, a procedure commonly employed by anthropologists, archaeologists, and forensic experts. The precision of age assessment is higher in young children, given the varied developmental stages of multiple teeth and other skeletal maturity factors. However, in adolescents and young adults, with only third molars developing, alternative age assessment methods become questionable, as sutures and epiphyses have already fused, and secondary sex characteristics have emerged by the middle teens and early twenties¹.

The 14-16 age group is important in medicolegal cases involving sexual offenses, juvenile offenders, kidnapping, and child labor. In all of these cases, a medical opinion on the victim's or accused's age may be requested. However, it is impossible for a forensic practitioner to determine an exact age. Instead, they can only estimate a range of ages². In such cases medical opinion regarding age of the victim or the accused is sought by officials. It is not possible to give exact age by medical examination. Higher Courts have held that one can estimate only a range of age³.

There is no single reliable factor, which alone can predict the exact age of an individual. A fairly close estimate range of age can be achieved by using multiple age indicators like ossification status, dental eruption and physical examination as compared to studying them individually.

Pubertal period in girls is accompanied by certain well-defined changes, which can be used to estimate the age. Determination of age with reasonable narrow range would include 1) Physical examination such as height, weight, general configuration and development of secondary sexual characters. 2) Number and nature of teeth as to mixed or otherwise in each jaw.

Teeth are known to aid in personal identification and age estimation as they are highly durable and resist putrefaction, fire, chemicals etc. The times of eruption of temporary and permanent teeth are fairly constant and assessment of age of an individual by examination of teeth is one of the accepted methods of age estimation.

The studies by various workers show that the factors like race, geography, climate, diet, heredity and endocrine factors do affect the physiological changes occurring at puberty. Appreciable variations have been recorded not only by workers from different countries but also by the workers from various provinces of Indian subcontinent.

So it becomes an obvious necessity to have a local data for each population so that a doctor determining age and attending the court of law can give a fairly accurate range of age, thus helping in administration of justice.

Through this study an attempt is made to know the status of ossification at elbow and wrist joints, dental eruption status and development of secondary sexual characters in school girls between 14 -16 years age group in Bagalkot City Schools

METHODOLOGY

The study received ethical approval from the Institutional Ethics Committee. After explaining the study's purpose and procedures, informed consent was obtained from the participants. A detailed questionnaire was used to gather relevant information from the subjects. This was done using the Simple Random Sampling technique. The study involved 100 girls between the ages of 14 and 16 from Basaveshwar English Medium High School in Bagalkot. These subjects were further divided into two groups: 50 girls aged 14 and 50 girls aged 15.

To qualify for the study, participants had to be healthy, normal girls between the ages of 14 and 16. They were also required to provide documentary proof of their age, such as a birth certificate from the municipal authority or school records. Additionally, they had to have been born and raised in the Bagalkot district. Participants were excluded from the study if they had skeletal deformities, diseases, malformations, or injuries. Individuals with severe malnutrition, endocrine disorders, or chronic illnesses were also excluded. Objective of the study was, to study the status of dental eruption in school girls of age group between 14-16 years in Bagalkot city and to study development of secondary sexual characteristics in school girls of age group between 14-16 years in Bagalkot city.

Method of Collection of Data

The 100 selected subjects were divided into two groups: the 14 years age group (14 to 15 years) and the 15 years age group (15 to 16 years). Each individual was assigned a unique code number from 1 to 100, ensuring that both the proforma. Informed consent was obtained from all participants after explaining the study's purpose and procedures. A questionnaire was administered to gather information on dietary habits, socio-economic status, and exercise habits, with socio-economic status determined using the Modified B.G. Prasad classification⁴.

Physical Examination

A standardized height measuring instrument was employed to measure height in centimetres, with subjects standing erect, heels together, shoulders, buttocks, and heels in contact with the scale, and looking straight. Weight was determined in kilograms using a standardized weighing machine without footwear. To assess the appearance and development of secondary sexual characteristics, subjects were examined in a private room with a cubicle curtain.

Dental Examination

The oral cavity was examined with mouth wide open with the help of a torch light. Detailed dental examination was done and findings were noted in the form of number of teeth erupted and any abnormalities in dentition.Space behind second molar tooth was assessed by putting a gloved index finger over the gingival area just behind the tooth and was inferred to be present in cases where hard part of underlying bone was appreciated and was inferred to be absent in cases where only soft tissue was felt.

Staging of second molar tooth eruption was done in the following manner:

- Stage 0 Non cutting through gum
- Stage 1 When tip of crown of tooth penetrated the gum margin.
- Stage 2 When this crown has grown into oral cavity beyond gum margins but not yet reached the occlusal plane.
- Stage 3 When the occlusal surface came in contact with its counterpart and the bite was complete.

Statistical analysis

The collected findings were organized into tables and subjected to statistical analysis using the SPSS software version 17.0. To enhance the interpretability of the results, comparisons were made with similar studies conducted in different regions of India and abroad. The statistical methods employed for analysis included percentages, mean calculations, the Chi-square test, and Fisher's exact test. These methods aimed to derive meaningful insights and identify any significant patterns or associations within the data.

RESULTS

For the purpose of comparison and analysis, equal number of females in each age group of 14 years (14 yrs+364 days) and 15 years (15 yrs+364 days) were selected for the present study. the distribution of study subjects according to socioeconomic status (SES), using the Modified B.G. Prasad Classification. The classification system divides the population into five classes, with Class I being the highest SES and Class V being the lowest SES. The majority of the study subjects (49%) belonged to Class II SES, followed by Class III SES (24%). Class I SES accounted for 22% of the subjects, and Class IV SES accounted for 25%. There were no subjects in Class V SES. In our study maximum (62%) of them were having mixed diet and 38% were vegetarian.

| Second Molar Eruption | 14-15 Years Girls | | 15 -16Y | ears Girls | Total | | | |
|--------------------------|---|-------------|---------|---------------|-------|-----|--|--|
| | Ν | % | Ν | % | n | % | | |
| Right Maxillary quadrant | uadrant Chi square test=1.04 p=0.30 | | | | | | | |
| Stage 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| Stage 1 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| Stage 2 | 3 | 6 | 1 | 2 | 4 | 4 | | |
| Stage 3 | 47 | 94 | 49 | 98 | 96 | 96 | | |
| Total | 50 | 100 | 50 | 100 | 100 | 100 | | |
| Left Maxillary quadrant | Left Maxillary quadrant Chi square test=1.04 p=0.30 | | | | | | | |
| Stage 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| Stage 1 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| Stage 2 | 3 | 6 | 1 | 2 | 4 | 4 | | |
| Stage 3 | 47 | 94 | 49 | 98 | 96 | 96 | | |
| Total | 50 | 100 | 50 | 100 | 100 | 100 | | |
| Left Mandibular quadrant | Chi squ | uare test=1 | .04 p | =0.30 | | | | |
| Stage 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| Stage 1 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| Stage 2 | 3 | 6 | 1 | 2 | 4 | 4 | | |
| Stage 3 | 47 | 94 | 49 | 98 | 96 | 96 | | |
| Total | 50 | 100 | 50 | 100 | 100 | 100 | | |
| Right Mandibular quadrar | nt Chisq | uare test= | 1.04 p |)=0.30 | | | | |
| \Stage 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| Stage 1 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| Stage 2 | 3 | 6 | 1 | 2 | 4 | 4 | | |
| Stage 3 | 47 | 94 | 49 | 98 | 96 | 96 | | |
| Total | 50 | 100 | 50 | 100 | 100 | 100 | | |

Table 1 presents In the upper and the lower jaw, on both right and left sides, second molar was seen in Stage 3 of eruption in 94% of cases of 14 years age group. This percentage increased to 98% in 15 years of age group. Stage 0 and Stage 1 of second molar eruption were not seen in any case. The two age groups are found to be similar with respect to eruption status as shown by p value= 0.30 in all four quadrants, so we can conclude that completion of second molar eruption is more likely to occur in 14-15 years of age group.

| | 14-15 Years Girls | | 15-16 Y | ears Girls | Total | | |
|--------------------------|--------------------------|-------------|------------|---------------|--------|-----|--|
| | n | % | N | % | n | % | |
| | Right Maxillary quadrant | | Chi squa | are test=6.25 | p=0.01 | | |
| Absent | 38 | 76 | 26 | 52 | 64 | 64 | |
| Present | 12 | 24 | 24 | 48 | 36 | 36 | |
| Total | 50 | 100 | 50 | 100 | 100 | 100 | |
| | Left Maxilla | ry quadrant | Chi squa | re test=6.25 | p=0.01 | | |
| Absent | 38 | 76 | 26 | 52 | 64 | 64 | |
| Present | 12 | 24 | 24 | 48 | 36 | 36 | |
| Total | 50 | 100 | 50 | 100 | 100 | 100 | |
| Right Mandibular quadran | | | nt Chisqua | are test=6.13 | p=0.01 | | |
| Absent | 19 | 38 | 8 | 16 | 27 | 27 | |
| Present | 31 | 62 | 42 | 84 | 73 | 73 | |
| Total | 50 | 100 | 50 | 100 | 100 | 100 | |
| | Left Mandibular quadrant | | | are test=5.17 | p=0. | 03 | |
| Absent | 18 | 36 | 10 | 20 | 28 | 28 | |
| Present | 32 | 64 | 40 | 80 | 72 | 72 | |
| Total | 50 | 100 | 50 | 100 | 100 | 100 | |

Table 2: Space behind Second Molar Tooth

Table 2 shows In 14 years age group, there was space behind second molar tooth on both right and left maxillary quadrant (upper jaw) only in 24% of cases. In the 15 years age group space behind second molar tooth was present in 48% cases in both right and left maxillary quadrant.

Considering the lower jaw (right and left mandibular quadrant), space behind second molar tooth was developed in 62% on right quadrant and 64% on left quadrant in case of 14 years girls. In the age group of 15 years, 84% of girls showed presence of space behind second molar tooth on right quadrant while on the left quadrant it was seen in 80% of girls.

The two age groups are found to be significantly different with respect to development of space behind second molar in both upper jaw with p=0.01 and lower jaw with p=0.01 and p=0.03 of right and left mandibular quadrant respectively.

In our study subjects, space behind second molar was seen to be developed more in lower jaw (73% in right lower quadrant and 72% in left lower quadrant) as compared to upper jaw (36% in both right upper and left upper quadrant).

| Pubic Hair Staging | 14-1 | 5 Years Girls | 15-16 | Years Girls | Total | | |
|--------------------|------|---------------|-------|-------------|-------|------|--|
| | n | % | n | % | N | % | |
| Stage 1 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Stage 2 | 8 | 16 | 2 | 4 | 10 | 10 | |
| Stage 3 | 17 | 34 | 15 | 30 | 32 | 32 | |
| Stage 4 | 24 | 48 | 30 | 60 | 54 | 54 | |
| Stage 5 | 1 | 2 | 3 | 6 | 4 | 4 | |
| Total | 50 | 100 | 50 | 100 | 100 | 100% | |

Table 3: Pubic hair staging in Girls

Table 3 shows Most of the girls from both the age group had Stage 4 (54%) of pubic hair growth, 34% girls of 14 years age had Stage 3 while 30% girls from 15 years age group had developed Stage 3. Only one girl of 14 years and 3 girls of 15 years had developed adult like (Stage 5) pubic hair. Out of 100 girls, 10% had developed Stage 2 followed by 32% had developed Stage 3.

There is no statistically significant association of age, exercise, diet and socioeconomic status on the development of pubic hair among girls with p=0.14, p=0.68, p=0.08 and p=0.38 respectively.

| Breast development Staging | 14-15 Years Girls | | 15-16 | Years Girls | Total | |
|----------------------------|-------------------|-----|-------|-------------|-------|-----|
| Bleast development Staging | Ν | % | n | % | N | % |
| Stage 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Stage 2 | 17 | 34 | 10 | 20 | 27 | 27 |
| Stage 3 | 26 | 52 | 16 | 32 | 42 | 42 |
| Stage 4 | 6 | 12 | 18 | 36 | 24 | 24 |
| Stage 5 | 1 | 2 | 6 | 12 | 7 | 7 |
| Total | 50 | 100 | 50 | 100 | 100 | 100 |

Table 4: Breast Development staging in Girls

Table 4 presents the above table depicting breast development stages in females denotes that, majority of girls from age group of 14 years had Stage 2 and Stage 3 in 34% and 52% respectively. Majority of 15 years girls had Stage 3 and Stage 4 in 32% and 36% respectively.

As a total in the study group 42% girls had Stage 3; followed by 27% with Stage 2 and 24% with Stage 4. Only 7% girls had Stage 5 of breast development.

| | Breast development staging in Girls | | | | | | |
|-------------|-------------------------------------|-------------|---------|---------|---------|-------|--|
| | Stage 1 | Stage 2 | Stage 3 | Stage 4 | Stage 5 | Total | |
| Age | | | | | | | |
| 14-15 years | 0 | 17 | 26 | 6 | 1 | 50 | |
| 15-16 years | 0 | 10 | 16 | 18 | 6 | 50 | |
| Total | 0 | 27 | 42 | 24 | 7 | 100 | |
| | Chi squa | are test=13 | 3.7 | p=0.003 | | | |
| Exercise | | | | | | | |
| Never | 0 | 24 | 40 | 23 | 6 | 93 | |
| Daily | 0 | 3 | 2 | 1 | 1 | 7 | |
| Total | 0 | 27 | 42 | 24 | 7 | 100 | |
| | Chi squa | re test=0.7 | 8 | p=0.85 | | | |
| Diet | | | | | | | |
| Vegetarian | 0 | 7 | 12 | 17 | 2 | 38 | |
| Mixed | 0 | 20 | 30 | 7 | 5 | 62 | |
| Total | 0 | 27 | 42 | 24 | 7 | 100 | |
| | Chi squa | re test=0.3 | 51 | p=0.95 | | | |
| Socioecono | mic Status | | | | | | |
| Class I | 0 | 7 | 9 | 5 | 3 | 24 | |
| Class II | 0 | 18 | 20 | 10 | 1 | 49 | |
| Class III | 0 | 1 | 13 | 6 | 2 | 22 | |
| Class IV | 0 | 1 | 0 | 3 | 1 | 5 | |
| Class V | 0 | 0 | 0 | 0 | 0 | 0 | |
| Total | 0 | 27 | 42 | 24 | 7 | 100 | |
| | Chi square test=1.707 p=0.19 | | | | | | |

 Table 5: Factors affecting Breast development staging in Girls

Table 5 shows Above table shows statistically significant difference of age in development of breast with p=0.003 while there is no significant statistical association of exercise, diet and socioeconomic status over development of breasts among girls with p=0.85, p=0.95 and p=0.19 respectively. As there are no representatives in Stage 0 and Socioeconomic status Class V, they are not included in statistical analysis. In 14 years age group 86% girls had Stage 2, and 8% had stage 3. While in 15 years

age group 14% had Stage 3 of axillary hair development. In the whole study population axillary hair had not appeared in 6% of girls and 11% of girls had developed (Stage 3) Adult type.

There is no significant statistical association of age, exercise, diet and socioeconomic status over axillary hair development among girls with p=0.14, p=0.4, p=0.20 and p=0.11 respectively. As there are no representatives in Socioeconomic status Class V, it is not included in statistical analysis.

| Menarche | | 14-15 years Girls | | 15-16 years Girls | | Total | |
|------------------|---------------|-------------------|-----|-------------------|-----|-------|-----|
| wenarche | | n | % | n | % | Ν | % |
| Not attained | | 0 | 0 | 0 | 0 | 0 | 0 |
| Attained | <1 years ago | 5 | 10 | 1 | 2 | 6 | 6 |
| | 1-2 years ago | 13 | 26 | 4 | 8 | 17 | 17 |
| | 2-3 years ago | 29 | 58 | 16 | 32 | 45 | 45 |
| | >3years ago | 3 | 06 | 29 | 58 | 32 | 32 |
| Total | | 50 | 100 | 50 | 100 | 100 | 100 |
| Mean age (years) | | 12.25 | | 12.43 | | 12.34 | |

 Table 6: Menarche in girls

Table 6 shows Menarche was attained by 58% of girls 2-3 years ago (12 yrs) and 26 % of girls 1-2 years (13 yrs) ago by girls of 14 years age. 58% of 15 years girls had attained menarche more than 3 years ago (12yrs). 4 girls had attained menarche 4 years ago that is at around 11 years of age. All the girls had attained menarche, and mean age of attainment of menarche was 12.25 years in girls of 14-15 years and 12.43 in girls of 15-16 years and for the entire study group it was 12.34 years. There is statistically significant difference of age in attainment of menarche with p=0.0001, while there is no significant statistical association of exercise, diet and socioeconomic status with attainment of menarche with p=0.53, p=0.17 and p=0.234 respectively. As there are no representatives in category of not attained and Socioeconomic status Class V, they are not included in statistical analysis.

DISCUSSION

The word eruption refers to the cutting of teeth through the gums. There is significant time lag between the cutting of the tooth in to the oral cavity and completion of eruption (i.e. completion of bite). The stages of eruption of second molar and third molar tooth can act as valuable tool for age estimation when used in combination with radiological and physical examination.

In the present study as shown second molar tooth was erupted in all the subjects of study group and among them 94% in the upper jaw and in the lower jaw had complete eruption in 14-15 year age group. Second molar had erupted in all girls (100%) in both upper and lower jaw in girls of 15-16 years. In remaining 6% of the study group second permanent molar was at stage 2 of eruption in all four quadrants of the jaw.

There was no significant difference with respect to eruption status between the two age groups as shown by p value=0.30 in all four quadrants, so we can conclude that complete eruption of second molar is more likely to occur in 14-15 years age group.

Similar study done by Shourie⁵ in Madras and Lahore children, he found that eruption of second permanent molar in 14-15 year aged girls was 95% in the upper jaw and 100% in the lower jaw, where as it was erupted in all girls (100%) of 15-16 years and is consistent with our study.Study by Kamalanathan et al⁶ in Siamese Village observed

that there was complete eruption of second molar in all girls (100%) by the age of 14 years.

Ingle D⁷ in Bijapur and Gulbarga mentions the average age of eruption of second molar as 14 years in 100% of females.

We could not compare our observation regarding second permanent molar eruption with that of Kishore U⁸ because of the limitation of the age group of our study group which is 14-16 years where as in study done by Kishore U⁸ the study group was of the age 10 to 20 years.

Modi⁹ mentions that a note should always be made as to whether there is a space behind second molar teeth if third molars are absent.

After the eruption of second permanent molars usually at 12-14 years; third molars do not erupt until age 17 years or later. Considerable posterior jaw growth is required after the age of 12-14 years to allow room for these teeth. This growth of jaw produces space behind second molar tooth which is appreciable between 14-16 years of age or later.

In the present study girls belonging to 14-15 years age group, 34% and 52% girls had Stage 2 and Stage 3 of breast development respectively, while 2% of the girls had Stage 5. Breast development was observed to be in Stage 2 and 3 among 20% and 32% of the girls respectively belonging to age group 15-16 years, while 36% had Stage 4 and only 12% had Stage 5(Adult type) development.

In the study by Sun¹⁰⁻¹³, Mexican American girls of 14-15 years had Stage 4 which is consistent with our observations, while white and African American girls had comparatively advanced development with Stage 5 in girls of age around 16 years. Breast development among these girls was seen to be in Stage 4 and 5 and hence more advanced as compared to findings of present study where it is Stage 2 and 3 in 14-15 years and Stage 3 and 4 in 15-16 years respectively.

Work by Choudhury¹⁴⁻¹⁷ in Orissa, found that 48.6% and 25.7% girls belonging to the age group 14-15 years had Stage 3 and Stage 4 respectively and only 14.3% had Stage 5 of breast development. While in the girls of age group between 15-16 years, 27.1% and 44.7% had Stage 3 and Stage 4 of Breast development respectively and 31.6% girls showed Stage 5 (Adult type) of breast development. None of the girls had Stage 2. Development of breast is 1 year earlier as compared to our study.

In the present study group 34% girls were in Stage 3 and 48% girls had Stage 4 of pubic hair development in the girls of age group between 14-15 years. Among the girls aged between 15-16 years, 30% had Stage 3 and 60% had Stage 4 of pubic hair development. In the present study majority of girls in the age group between 14-16 years were in between Stage 3-4 of pubic hair development.

Axillary hair had appeared in 94% of the girls by the age of 14-15 years in present study, and in all the girls belonging to age group between 15-16 years. Hence axillary hair appears by 14-15 years in girls of this region.

In the present study, the mean age of menarche was 12.34 years. Another study by Lal R and Townsend^{18_20} in Lucknow observed that age of menarche in majority of girls was 12-14 years, and is consistent with our study.

CONCLUSIONS

Complete eruption of the second molar occurred in 96% of girls aged 14-15 and in all girls aged 15-16. Additionally, noticeable development of the space behind the second molar in the lower jaw was observed by the age of 15-16 in females. In the age group of 14-16 years, most females exhibit Stage 3 or Stage 4 of pubic hair development. Breast development ranged from Stage 2-3 in 14-15-year-olds to Stage 3-4 in 15-16-year-olds. The mean age of menarche was 12.34 years. It's worth noting that pubertal changes are not influenced by socioeconomic status, exercise, or diet.

Limitation

This study was limited by its small sample size, which means that the results may not be generalizable to the wider population. Large scale studies from whole of Karnataka and India are needed to undertake Therefore, it is important to replicate this study in other settings with larger samples to confirm the findings.

Ethical Approval

This study was conducted in accordance with the Declaration of Helsinki-Ethical principle for medical research involving human subjects. Accordingly, the ethical clearance was obtained from a joint ethical review committee intuitional ethical committee KLE JGMM Medical College, KLE Academy of Higher Education and Research, Hubli, Karnataka. All individuals who took part in the study gave their informed consent, and data confidentiality was ensured.

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Authors' contributions

Dr Rekha rani Kumbar - conceptualization, data curation, investigation, methodology, project administration, visualization, writing—original draft, writing—review and editing; *Dr.S.Nandakumar* and *Dr. M. Nithya* -conceptualization, methodology, writing—original draft, writing—review and editing; *Dr. Priyanka Murgod* and *Dr Doddabasappa S Belavagi* - conceptualization, visualization, supervision, writing—original draft; *Dr.Mallikarjun.S Ballur(MSB)* - methodology, writing—original draft, writing, review and editing. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work. All authors have read and agreed to the published version of the manuscript.

Data Availability

All datasets generated or analyzed during this study are included in the manuscript.

Informed Consent

Written informed consent was obtained from the participants before enrolling in the study.

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Conflicts of interest:

There are no conflicts of interest.

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