Medical University -Varna

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Faculty „Dental Medicine”

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Evaluation of individual indicators for orthodontic treatment of children with mixed and permanent dentition

ABSTRACT

Of dissertation for the award of educational and scientific degree “PhD”

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Field of higher education:

7. Health and sports, Professional field: 7.2.DentalMedicine,

Scientific specialty: "Orthopaedic dentistry"

Supervisor:

Assoc. Prof. Dr Hristina Ivanova Arnautska, PhD

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The Dissertation contains 138 pages, 39 tables, 55 figures, 5 schemes and 16 application. 161 literary sources were cited, including 7 in Cyrillic and 154 in Latin.

The official defence of the Dissertation will take place on 28.07.2022. In Auditorius" Assoc. prof. Dr. Dimitar Klisarov " at the Faculty of Dental Medicine, "Prof. Dr. Paraskev Stoyanov" University of Varna in session of a scientific jury in the composition:

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The materials on the defence are available in the Scientific Department of MU - Varna and have been published on the website of the Medical University - Varna.

Note: In the abstract, the numbers of the tables and figures do not correspond to the numbers in the Dissertation.

CONTENTS:

I would like to express deep and sincere gratitude to my research supervisor Assoc. Prof. Dr Hristina Arnautska, PhD Head of Department of Orthodontics Faculty of Dental Medicine- Varna

LIST OF ABBREVIATIONS USED

DA	Dental age
DAD	Dental age of Demirjian
DAW	Dental age of Willems
OPG	Orthopantmogram
TRG	Telerenthgenogram
CA	Chronological age
CVM	Cervical vertebrae maturation
CS	Cervical stage
C2, C3, C4	Second, third and 4-th cervical vertebrae

I.Introduction

Knowing and understanding the nature of growth and development of maxillofacial structures in the norm and pathology, dynamics of these processes over the time influenced by environmental, genetic and epigenetic factors are essential for orthodontic treatment planning, prognosis and retention approaches. The period of facial growth spurt is significant for optimal treatment of malocclusions with orthopaedic effect as well its end allowing orthognathic surgery corrections. [11, 58, 51]

Evaluating the timing of growth movements, acceleration and amount is crucial for treatment of malocclusions in Orthodontics. [148]

During ontogenesis the physical changes of each individual is unique and the assessment of physiological processes are performed by evaluation of different tissues maturity like dental, somatic, skeletal and reproductive system. [147]

To evaluate the growth onset, spurt and deacceleration many somatic parameters are been investigated such as body height [76, 19, 122], body weight [64], chronological age (CA) [64, 67, 52], secondary sexual characteristics [68, 69].

Frontal sinus width [133], skeletal age, absence or presence of various osseous centres in hand and wrist [109, 53, 152, 129, and 150], cervical vertebrae maturation morphological indicators [12, 91, 71, 13], tooth eruption and mineralization stages [39, 118, 42] were also been investigated. The most contemporary methods examine saliva and serum biomarkers such as insulin like growing factor, growth hormone, alkaline phosphatase and creatinine. [56, 9, 79, 67, 125, 78, 148, 116, 81]

The chronological age is accepted as the most inaccurate factor for growth evaluation, especially if used as a sole indicator. [147]

From orthodontic point of view, the most useful methods of individual growth and developmental potential assessment are those easily applied on regular orthodontic roentgenographs such as orthopantomograms (OPG) and teleoroentgenograms

(TRG), with high accuracy for the purposes of treatment process.

II. PURPOSE AND TASKS

Purpose:

The aim of the Dissertation is by examining individual indicators of the pubertal growth period to create a prognostic model on orthopanthogram for the initiation of orthodontic treatment.

To fulfill the goal thus formulated, we set the following tasks:

Task 1

To conduct a study to assess the dental age of children in mixed and permanent dentition aged 7-16 years by using the methods of Demirjian [39] and Willems [160] To determine the accuracy of each method (up to 0.5 years) relative to the chronological age and between them.

Task 2

To determine the mean chronological age of the onset of pubertal growth peak defined by the method of cervical vertebrae maturation.[13]

Task 3

To determine the mineralization stages of mandibular canine (33), mandibular second premolar (35), mandibular second molar (37) and mandibular third molar (38) in the left lower quadrant, as well as maxillary left canine (23) relative to the stages of maturation of the cervical vertebrae .

Task 4

To determine the correlation between the stages of mineralization of the teeth examined in Task 3 and the stages of maturation of the cervical vertebrae CVM II, CVM III, CVM IV of Baccetti method, by specifying

indicators of the puberty development on the orthopantomogram and to provide guidance for the initiation of orthodontic treatment.

III.MATERIAL AND METHODS

MATERIAL

For the purposes of this dissertation 320 children, outpatients in the Faculty of Dental

Medicine at the Medical University "Prof. Dr. Paraskev Stoyanov" Varna, Department of Orthodontics were examined. The study has a positive assessment by the Committee on The Ethics of Research at the Medical University " Prof. Dr. Paraskev Stoyanov"- Varna, Bulgaria according to Protocol 107/28.10.2021
The selection of children was carried out according to the following criteria:

1. Chronolocical age 7-17 years
2. Mixed or permanent dentition
3. Without accompanying systemic diseases
4. Without hypodontia and/ or extractions of permanent teeth in the lower jaw
5. Without retinated and/or ankylosed teeth in the lower jaw
6. Without facial injuries
7. Outpatients
8. No orthodontic treatment performed

All ortopantomograms (OPG) and lateral telerentgenograms (TRG) studied - a total of 640 were digital. Imaging 2D diagnostics were performed with a Planmeca ProMax 2D apparatus (Planmeca OY, Asentajankatu 6, FIN-00880 Helsinki, Finland) -a system for maxillofacial imaging in the Dental Medicine Faculty ofVarna. Patented SCARA technology guarantees clear,

unmistakable, anatomically accurate image geometry.Exposition time-
OPG- 16 sec, 13 mA, 68 KV,
TRG-6.7 sec, 9 mA, 65 kV.
The visualization of radiographs is carried out using ROMEXIS viewer software, allowing image enlargement if necessary for greater accuracy of the study and AudaxCeph-Cephalometric x-ray analysis software-for TRG analysis. The results of each child's surveys were evaluated according to 19 indicators recorded in a statistical card. The main measurements for this Dissertation work are a total of 6129 in number, task 1 being 2065, task 2 is 960, the third task is 1552 and task 4 is 1552 respectively.

Material Task 1

In the performance of the first task, 295 opropanthomograms of children in mixed and permanent dentition aged 7.00-16.00 years were examined, of which 115 (39%) boys and 180 (61%) girls (Fig. 1)

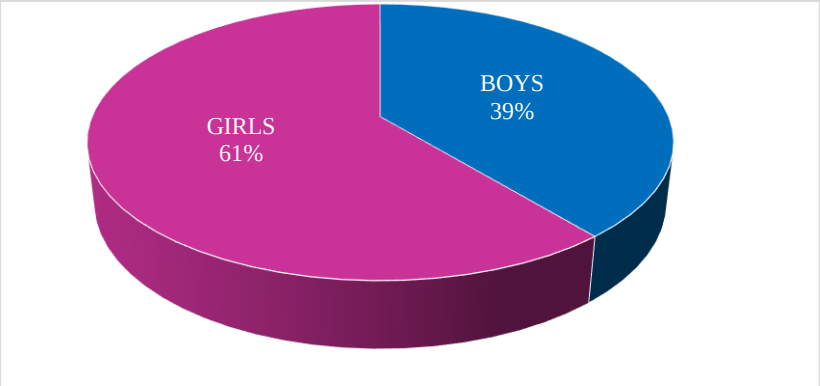


Figure 1: Distribution as a percentage of the surveyed children for both genders

The children studied were divided into four age groups (table 1) based to the period of development of dentition, according to the classification of Van der Linden [158]

Age group	Boys		Girls		Total	
	N	%	N	%	N	%
7,00-9,99	23	45.1%	28	54.9%	51	100%
10,00-11,99	40	42.6%	54	57.4%	94	100%
12,00-13,99	32	32.3%	67	67.7%	99	100%
14,00-15,99	20	39.2%	31	60.8%	51	100%
Total	115	39%	180	61%	295	100%

Table 1: Distribution of subjects studied by number, gender and age in four groups

Material task 2

In the performance of a second task, 320 telegengenograms were examined , of which 122 of boys and 198 of girls. (Fig. 2)

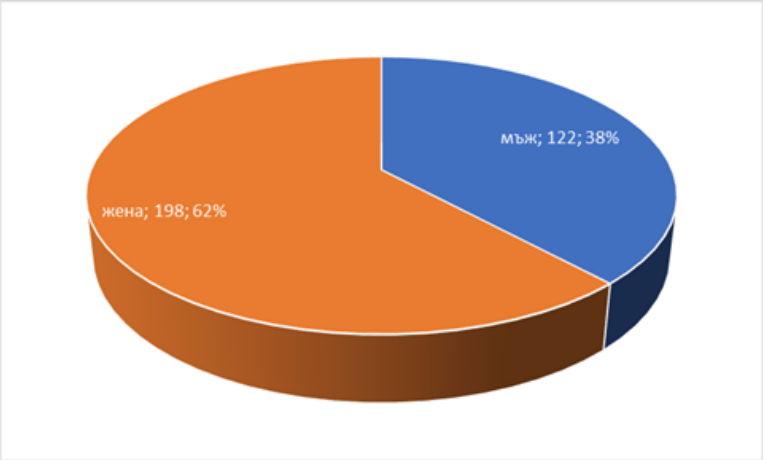


Figure 2: Distribution as a percentage of the surveyed children for both genders

Included children were aged 7 to 17 years, divided into 11 age groups over a period of 12 months increment (Fig. 3)

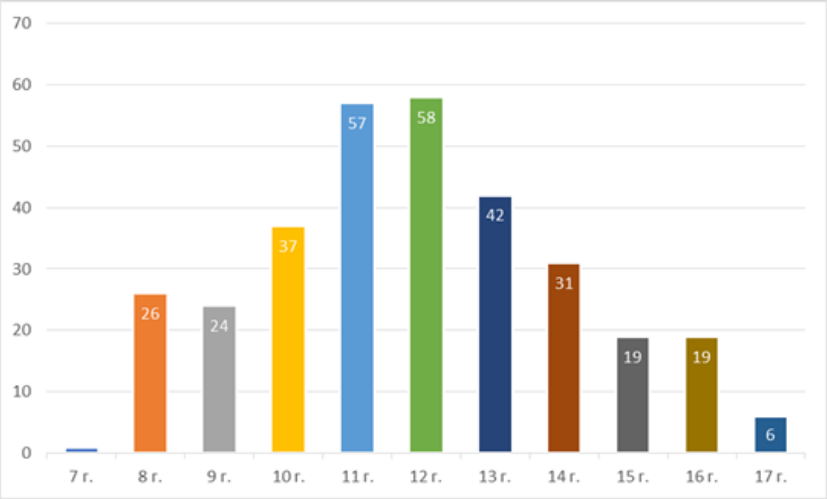


Figure 3: Age structure and number of children surveyed

Material task 3

In the performance of a third task, we assigned three target groups – in the first group children with cervical vertebrae maturation stage CVM II were included, in the second group subjects in stage CVM III were included and in the third group, children in the CVM IV stage were selected. After examining 320 telegengenograms in the first group, we included 64 children of which 38 boys and 26 girls. In the second group we included 74 children, of which 18 boys and 56 girls, and in the third group 56 children - 13 boys and 43 girls respectively - a total of 194 children. (Table 2)

CVM	Gender	N	Total
CVM II	boys	38	64
	girls	26	
CVM III	boys	18	74
	girls	56	
CVM IV	boys	13	56
	girls	43	
Total	boys	69	194
	girls	125	

Table 2: Target groups including boys and girls in CVM II, CVM III and CVM IV

An OPG and a TRG were obtained for each child in the same visit. Out of 320 orthopantomographs, we selected 194 of them for the same children from the target groups and determined the degree of mineralization of the dental germ by the Demirjian method of left mandibular canine (33), left maxillary (23) canine, left mandibular second premolar (35), second left mandibular molar (37) and left mandibular third molar (38).

Material task 4

In the performance of a fourth task, we examined the correlation between the degree of mineralization of the teeth 23, 33, 35, 37, 38 determined by the Demirjian method of 194 OPG with the stages of maturation of the cervical vertebrae CVM II, CVM III, CVM IV of Baccetti method, defined at 194 TRG of the same children.

Method Task 1

In the performance of the Task 1, assessment of the dental age by the degree of mineralization and maturation of dental germs was done by the methods of Demirjian et al. [39] and Willems et al. [160] on digital OPG. The chronological age of each subject was calculated by subtracting the date of the orthopantomographic examination from the date of birth after converting both to a decimal age (rounded to two decimal places). The age was calculated by coding scheme-

By a quarter (.25) age between > 1 и ≤ 3 months

• With two quarters (.50) age between > 3 и ≤ 6 months

• With three quarters (.75) age between > 6 и ≤ 9 monts

Round numbers- the age in years.

On digital OPG one of the eighth developmental stages (A–H) of the seven teeth in the left mandibular quadrant were evaluated from the central incisor to the second molar was defined by both methods- Demirjian's (1973) [39] and Willems methods [160] Fig.4, fig.5

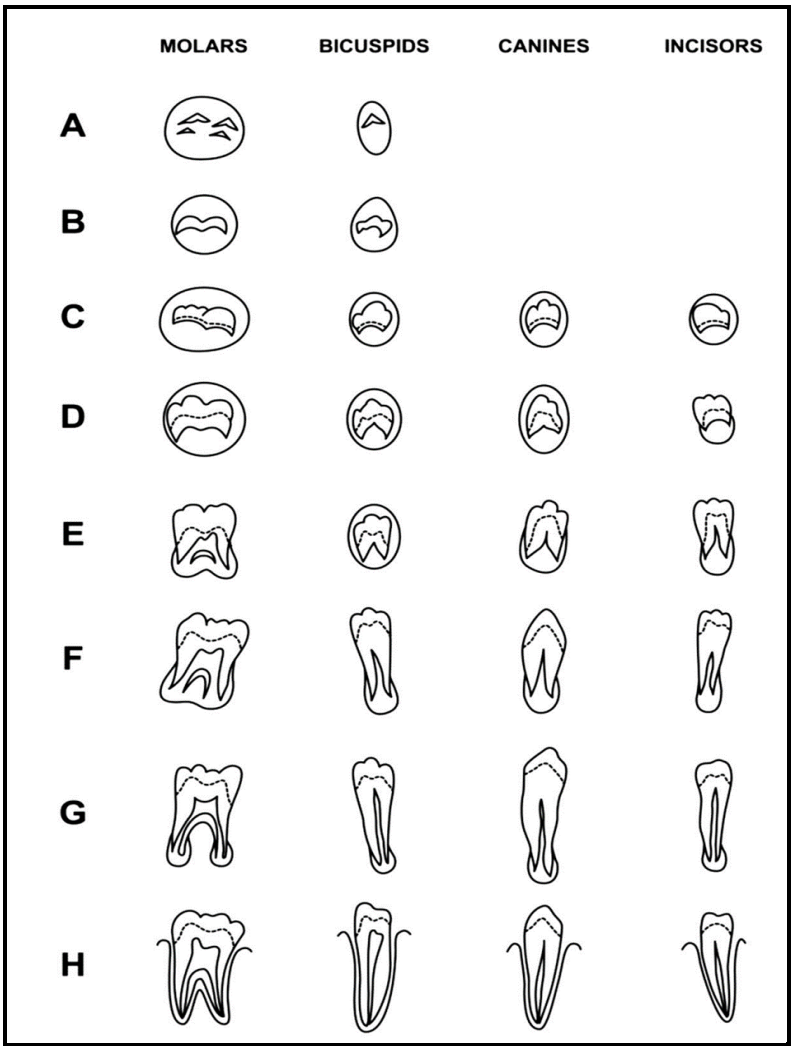


Figure 4: Schematic representation of the maturational stages of permanent single-rooted and multi-rooted teeth [39]

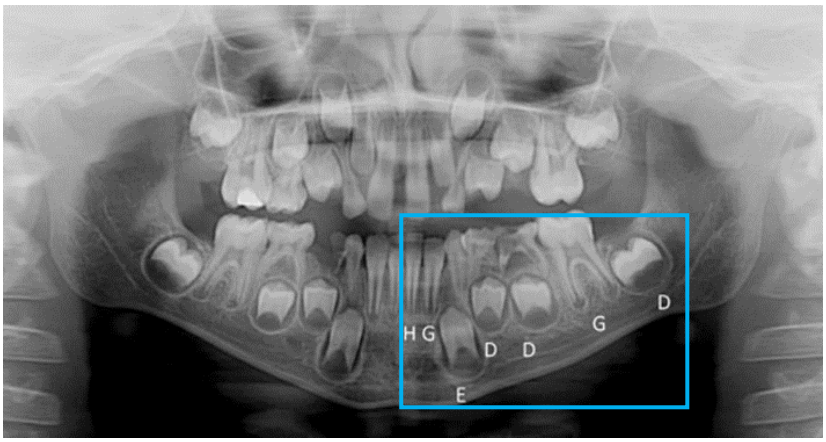


Figure 5: Permanent teeth in the lower left quadrant at different stages of calcification-early mixed dentition [80]

On OPG the degree of development and calcification of permanent teeth was performed. The left lower-jaw quadrant was evaluated from the central incisor to the second molar , starting from mineralization of occlusal tubercles without fusion of different zones (A), to the end of the root development with a closed apical foramen (H).

For each stage of dental development (A-H) one, two or three criteria (a, b, c) are included. If only one criterion is included, the stage shall be considered to be fulfilled if the dental development fully corresponds to that description. In the indication of two criteria, the stage shall be considered to have been reached if only the first criterion is met. For three specified criteria for the stage concerned, it shall be considered to be fulfilled if the first two criteria are satisfied. In addition to meeting these requirements to reach the relevant stage, all criteria for the preceding

stages must be met. In borderline cases, the earlier stage of maturation is always accepted. (Table 3)

A	Initial signs of mineralization of the dental crown, both in single-rooted and multi-rooted teeth in the form of cone-shaped nuclei, located at the top of the crypt. There is no merging between the different mineralisation points.
B	Presence of a fusion between mineralization points forming one or more tubercles, outlining the future occlusal surface of the tooth
C	а) The formation of enamel on the occlusal surface of the tooth is complited, with a visible converging towards tooth cervix. б) The deposition of coronary dentin is visible. в) The occlusal surface of the pulp chamber is outlined in the form of curvature.
D	а) The tooth crown is formed to the enamelo-cementum border б) The occlusal surface of the pulp chamber in single-rooted teeth has a clear curvature

	shape outline, concave towards the cervix.Pulp horns form umbrella-like shape of the pulp chamber. In multi-rooted teeth, the pulp chamber has a trapezoidal shape. в) The initiation of root development is visible in the form of spicules.
E	Single-rooted teeth: (a) The walls of the pulp chamber shall take the form of straight lines, the course of which is disturbed by pulp horns which are larger than the previous stage. b) The length of the newly formed root is less than the height of the dental crown Multi-rooted teeth: (a) Iniciation of root bifurcation formation of a characteristic semi-lunar shape or in the form of a point. b) The length of the newly formed roots is less than the height of the dental crown.
F	Single-rooted teeth:

	a) The walls of the pulp chamber form an isoscele triangle. The apical part of the root has a funnel-like shape. (b) The length of the forming root shall be equal to or greater than the height of the tooth crown. Multi-rooted teeth: (a) Root bifurcation is developed in length. Gives a characteristic funnel-shaped appearance to the apical part of the roots. (b) The length of the root forming is equal to or greater than the height of the tooth crown
G	The walls of the root canal are parallel, as the apical are partially open (distal root in molars)
H	a) The root apex is completely closed (the distal root in molars) b) The periodontium is of equal width around the entire root, including apex.

Table 3: Description of the degree of development of dental germs in stages(A-H) and the corresponding criteria for reaching it[39]

With aid of tables developed by Demirjian et al. [39] maturational points corresponding for each stage of mineralization of permanent teeth from the lower left quadrant for boys and girls were defined. The sum of the points for seven left teeth were converted to the dental age of the person studied.

In 2001, Willem's et al., [160] revised the Demirjian method and the assessment of the degree of mineralization of dental germs being maintained in the same eight stages (A-H) and under the same criteria, for each stage. The study shall be carried out at the OPG. Each phase of dental development corresponds to age points representing parts of dental age, separately for girls and boys. The sum of the points directly results in dental age.

Method Task 2

An assessment of skeletal age by the degree of morphological maturation of the second (C2), third (C3) and fourth (C4) cervical vertebrae-CVM (cervical vertebral maturation) was carried out on digital telegengenographies using the Baccetti et al method. [13]

Methodology for visually determining the morphological characteristics of the cervical vertebrae of lateral TRG was as follow:

On a lateral telegenerogram, the following points were indicated and tracked to assess the morphological characteristics of the bodies of C2, C3 and C4 vertebrae according to Baccetti's method. [13]

C2-second cervical vertebrae.

C2m- the deepest point of the lower border of the vertebral body C2a-anterior lower point of the vertebral body . C2p-posterior lower point of the vertebral body.

C3-third cervical vertebrae.

C3m- the deepest point of the lower border of the vertebral body .C3la- the anterior lower point of the vertebral body C3lp-posterior lower point of the vertebral body C3ua -the anterior upper point of the vertebral body. C3up- the posterior upper point of the vertebral body.

C4- fourth cervical vertebrae.

C4m- the deepest point of the lower border of the vertebral body. C4la-anterior lower point of the vertebral body C4lp-posterior lower point of the vertebral body . C4ua -the anterior upper point of the vertebral body. C4up - the back upper point of the vertebral body. (Fig. 6):

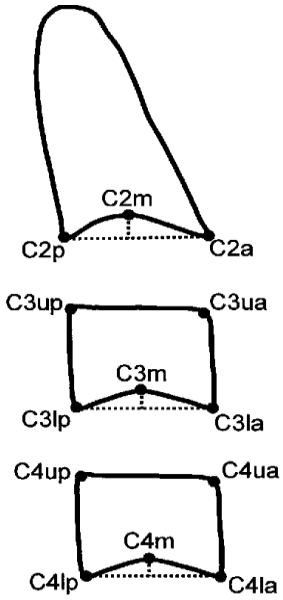
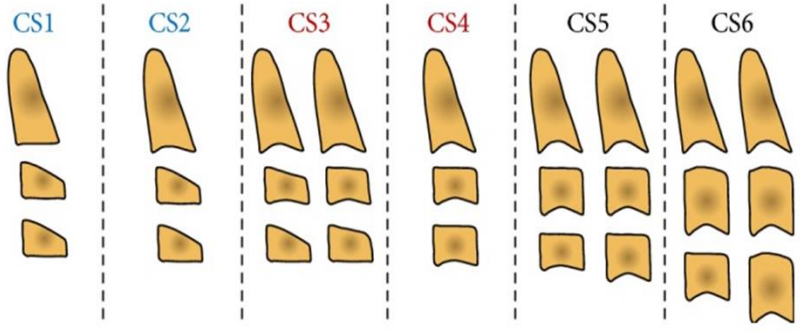


Fig. 6 : Morphological characteristics for the assessment of skeletal age by the degree of morphological maturation of C2, C3, C4. [13]

On the basis of the morphological characteristics described, six stages of cervical maturation (Fig. 7) are determined by the Baccetti et al.[13] and skeletal age is established.



CS2 Prepubertal stage CS3, CS4 Puberty peak CS5, CS6 Post-peak stage

Fig. 7: Schematic representation of the stages of maturation of cervical vertebrae. Different combinations of morphological characteristics of the bodies of C2, C3 and C4 cervical vertebrae **[51]**

Method Task 3

The degree of mineralization of target teeth -23, 33, 35, 37 and 38 by the Demirjian et al. method (1973)[39] in eight stages designated by A-H, described in task 1 methodology was determined.The results were compared with the stages of skeletal maturation CVM II, CVM III, CVM IV defined by the Baccetti et al method(2005) [13] described in task 2 methodology.

Method Task 4

In the performance of task 4, the correlation between the most common stages of mineralization of teeth-23, 33, 35, 37 and 38 by the Demirjian et al method (1973) [39] established in Task 3 with the stages of skeletal maturation-CVM II, CVM III, CVM IV by the Baccetti et al. method (2005) [13] defined in Task 2 was examined.

The correlation rate (r) was determined on a 5-graded scale. The numerical value of the coefficient – r can vary from 0 to 1. At r =1, the dependency is functional. The intermediate meanings of the coefficient- r are interpreted on the following 5-graded scale (table 4)

Interpretation	Degrees of r in 5-graded scale
Weak	Up to 0.3
Moderate	From 0.3 to 0.5
Strong	From 0.51 to 0.7
Very strong	From 0.71 to 0.9
Significant	Over 0.9

Table 4: Correlation rates for intermediate meanings of (r)

Statistical methods of analysis

The following statistical methods were applied:

1. Descriptive analysis – includes mean values, standard deviation, median and interquartyel range in quantitative variables; Summarize and organize characteristics of a data set- a collection of responses or observations from a sample or entire population.

2. Chi-square criterion method to compare proportions and frequency in data.

3. The Wilcoxon signed-rank test - a non-parametric statistical hypothesis test used either to test the location of a population based on a sample of data,

or to compare the locations of two populations using two matched samples.

4. Graphical analysis – to visualize the results obtained.

5. Variational analysis of quantitative variables– calculation of central trend and dissipation estimates, average, standard deviation.

6. Methods of Kolmogorov-Smirnov (Kolmogorov-Smirnov) and Shapiro-Wilk – to check the normality of the distribution of the quantitative variable.

7. Student T-test – to check hypotheses for difference between two dependent samples in statistically significant differences in quantitative indicators with normal distribution and for interval evaluation/confidence interval (CI) reflects the reliability of the assessment of the test parameter/.

8. Spearman correlation coefficient (r) to study the linear relationship between two dimensions, at least one of which was measured on an ordinal scale.

9. A p-value 0f <0.05 was considered statistically significant

*** Acknowledgments**

The author would like to thank Dr. Anzhela Bakhova for her great statistical assistance.

IV.RESULTS AND DISCUSSION

Results on Task 1:

Results for Age group-7.00-9.99 years

Continuous variables for normality the Kolmogorov-Smirnov test was used. As seen on Fig.8 and Fig.9 the test showed the normal distribution of data and parametric tests were required for analysis in this age group in both genders. The mean CA for male subjects was 8.78 ±0.65 years; mean DAD was 9.13±0.66 years, mean DAW was 9.04 ±0.6 years. The mean CA for female subjects was 8.93±0.60 years; DAD was 9.56±0.60 years, DAW- 9.09±0.87 years.

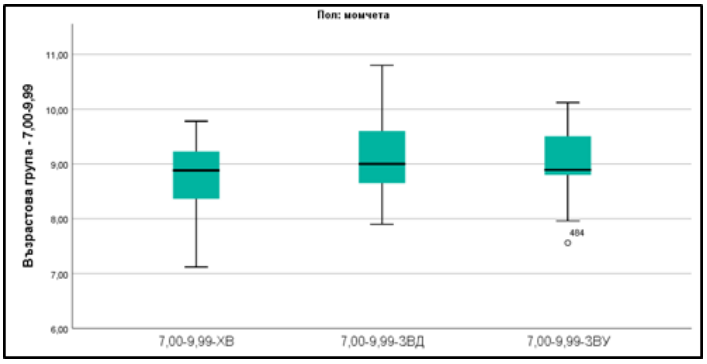


Fig. 8: box plot of CA; DAD; DAW in age group 7, 00-9, 99 for boys

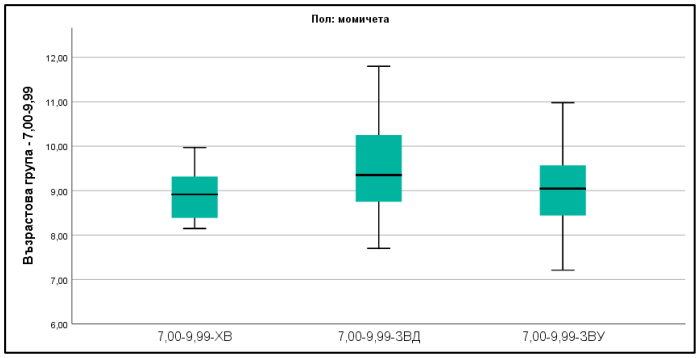


Fig. 9: box plot of CA; DAD; DAW in age group 7, 00-9,99 for girls

The hypothesis of no statistically significant difference ($p \leq 0.05$) between CA and DAD and CA- DAW was considered. With the t-test, the hypothesis was disclaimed for both methods DAD and DAW for boys and DAD for girls. The difference (CA-DAW) was not statistically significant ($p=0.13$) for girls so the conclusion that in this age group for female subjects the DAW estimate the CA correctly was done. Tabl.5

Age group	**Gender**	**N**	**Age**	**Mean**	**SD**	**t-criterion**	**P value**
7,00-9,99	boys	23	CA	8.78	0.65	4.425	0.000214
			DAD	9.13	0.66		
7,00-9,99	boys	23	CA	8.78	0.65	3.970	0.000645
			DAW	9.04	0.6		
Age group	**Gender**	**N**	**Age**	Mean	SD	t-criterion	P value
7,00-9,99	girls	28	CA	8.93	0.6	4.72	0,000064
			DAD	9.56	0.6		
7,00-9,99	girls	28	CA	8.93	0.6	1.56	0,129992
			DAW	9.09	0.87		

Tabl.5 Comparison of mean values of CA, DAD, DAW for both genders in age group 7.00-9.99 years

The next step in this study was to evaluate CA as overestimated or underestimated for those subjects and method, where the difference between CA and DAD or CA, and DAW was presented. The hypothesis of no difference between CA - DAD and CA- DAW was considered. With the Student's t-test, the hypothesis was disclaimed for DAD for both genders - for boys CA was overestimated by 0.18 -0.51 years, whereas the CA was overestimated by 0.35- 0.9 years for girls. DAW overestimated the CA for boys only by 0.12-0.4 year, whereas for girls the difference between CA and DAW was not statistically significant (p= 0.13). With this result we double verified the accuracy of DAW

method in this age group for female subjects. Tabl.6

Age group	Gender	N	Absolute difference	Mean Difference	SD	SE	95% Confidence Interval for Mean		t value	P value
							Lower Bound	Upper Bound		
7,00-9,99	boys	23	CA-DAD	-0.35	0.38	0.08	-0.51	-0.18	-4.42	0,000
			CA-DAW	-0.26	0.31	0.07	-0.4	-0.12	-3.97	0.001
	girls	28	CA-DAD	-0.63	0.7	0.13	-0.9	-0.35	-4.72	0
			CA-DAW	-0.15	0.51	0.1	-0.35	0.05	-1.56	0.13

Tabl. 6 Comparison of the absolute difference of CA-DAD and CA-DAW for both genders in age group

7.00-9.99 years

Age group	Gender	N	Mean CA-DAD	Mean Difference	SE	95% Confidence Interval for Mean		t value	P value
						Lower Bound	Upper Bound		
7,00-9,99	Boys	23	-0,35	0,28	0,15	-0,03	0,59	1,82	0,08
	Girls	28	-0.63						
			Mean CA-DAW						
	Boys	23	-0.26	-0,11	0,12	-0,36	0,14	-0,88	0,38
	Girls	28	-0.15						

Tabl. 7 Comparison of the absolute difference in measurements between CA-DAD, CA-DAW and gender in age group 7.00-9.99

No gender dependent correlation between the difference of CA-DAD (r=0,18; p=0.13) and CA-DAW (r=0.11; p=0.44) was found (tabl.7)

Results for Age group-10.00-11.99 years

Continuous variables for normality the Kolmogorov-Smirnov test was used. Graphically presented results of the test - Fig. 3 showed the normal distribution of data for male subjects but not for the female ones. The mean CA for boys was 11.29±0.52 years; mean DAD was 12.46± 1.05 years, mean DAW was 11.92 ±0.97 years. The mean CA for girls was 11.05 years (IQR-

10.62-11,62); DAD was 13.1 years (IQR 12.00-13.70); DAW- 12.27 years (IQR 11.24-12.88) Fig. 10 and Fig.11

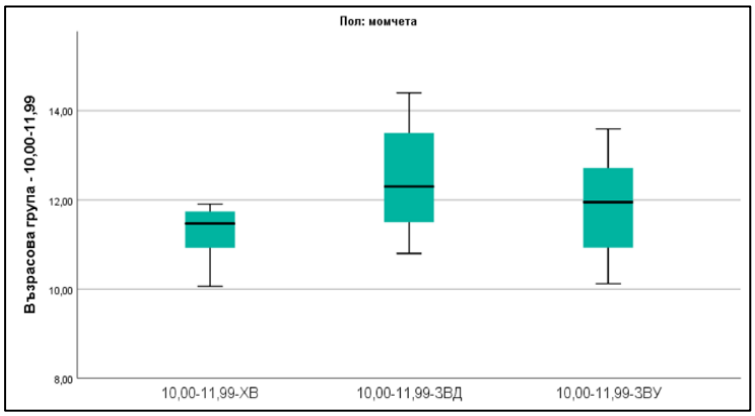


Fig. 10: box plot of CA; DAD; DAW in age group 7, 00-9, 99 for boys

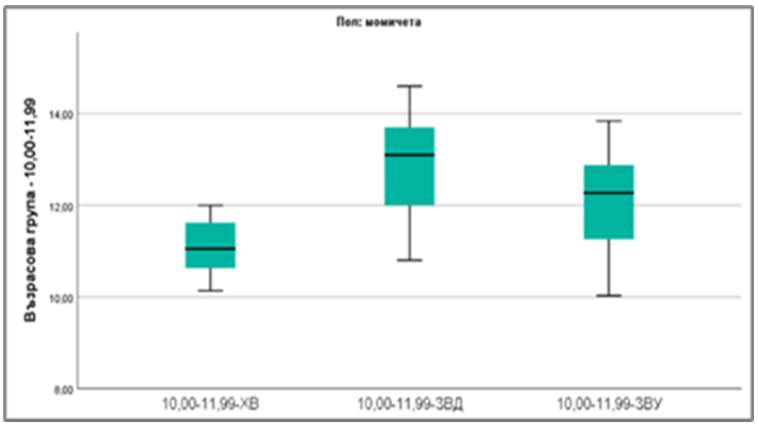


Fig. 11: box plot of CA; DAD; DAW in age group

7, 00-9, 99 for girls

The hypothesis of no statistically significant difference ($p \leq 0.05$) between CA and DAD and CA DAW was considered. With the t-test for boys (tabl.9) and Wilcoxon, signed-rank test for girls (tabl.8) the hypothesis was disclaimed for both methods for both genders.

Age group	**Gender**	**N**	**Age**	**Median**	**P value за Wilcoxon signed-rank test**
10,00-11,99	girls	54	CA	11.05	0,000
			DAD	13.10	
10,00-11,99	girls	54	CA	11.05	0,000
			DAW	12.27	
		N	**Age**	**Mean SD**	**T-criterion**
10,00-11,99	boys	40	CA	11.29 0.52	7.43
			DAD	12.46 1.05	
10,00-11,99	boys	40	CA	11.29 0.52	4.03
			DAW	11.92 1.02	

Tabl.8, Tabl.9 Comparison of mean values of CA, DAD, DAW for girls and boys in age group 10.00-11.99 years

The next step was to evaluate CA as overestimated or underestimated by DAD and DAW. The hypothesis of no difference between CA - DAD and CA- DAW was considered. With the Student's t-test, the hypothesis was disclaimed for DAD for both genders - for boys CA was overestimated by 0.9 -1.49 years, whereas the CA was overestimated by 1.55- 1.98 years for girls. DAW overestimated the CA for boys only by 0.31-0.94 year, whereas for girls the CA was overestimated by 0.74-1.06 years. -tabl.10

Age group	**Gender**	**N**	**Absolute difference**	**Mean Difference**	**SD**	**SE**	**95% Confidence Interval for Mean**		**t value**	**P value**
							Lower Bound	Upper Bound		
10,00-11,99	boys	40	CA-DAD	-1.17	0,99	0,16	-1,49	-0,9	-7,43	0.000
			CA-DAW	-0.63	0.99	0.16	-0.94	-0.31	-4.03	0.001
	girls	54	CA-DAD	-1.77	0.79	0.11	-1.98	-1.55	-16.47	0.000
			CA-DAW	-0.95	0.77	0.10	-1.16	-0.74	-9.09	0.000

Tabl.10 Comparison of the absolute difference of CA-DAD and CA-DAW for both genders in age group 10.00-11.99 years

No gender dependent correlation between the difference of CA-DAW (r=0,15; p=0.14) was found.

For Demirjian method we found gender dependent correlation between the difference CA-DAD (r=0.33; p=0.01).The conclusion that Demirjian method is more inaccurate for girls compared to boys in this age group was done (tabl.11)

Age group	Gender	N	Mean CA-DAD	Mean Difference	SE	95% Confidence Interval for Mean		t value	P value
						Lower Bound	Upper Bound		
10,00-11,99	boys	40	-1.17	0,60	0,19	0,21	0,97	3,14	0,003
	girls	54	-1.77						

Tabl. 11 Comparison of the absolute difference in measurements between CA-DAD, CA-DAW and gender in age group 10.00-11.99 years

Results for Age group-12.00-13.99 years

The Kolmogorov-Smirnov test indicated an abnormal distribution of the data in this age group for both

genders. Graphically the results are presented on Fig. 12 and fig. 13

The median of CA for boys was 12.58 years (IQR 12,18 - 13,07); DAD was 14.4 years (IQR 12,60 - 14,40); DAW - 13.59 years (IQR 12,38 - 13,59) . The median of CA for girls was 12.90 years (IQR-12.44-13,45); DAD was 13.7 years (IQR 13.7-14.60); DAW- 12.88 years (IQR 12.88- 13.84).

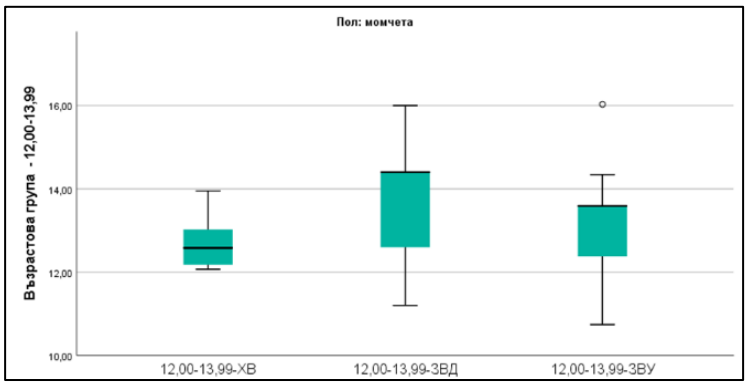


Fig.12 box plot of CA; DAD; DAW in age group 12, 00-13, 99 for boys

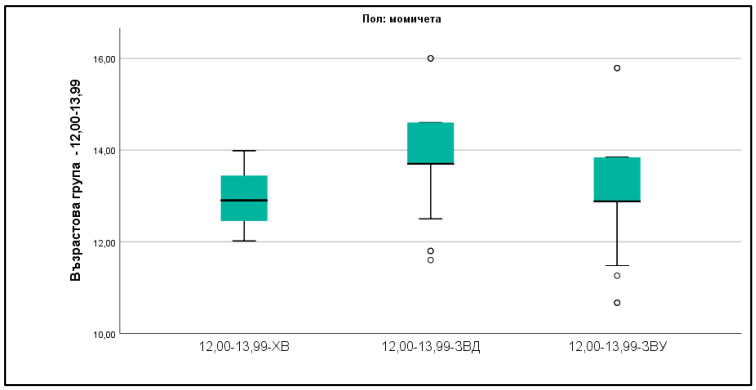


Fig. 13 box plot of CA; DAD; DAW in age group 12, 00-13, 99 for girls

The hypothesis of no statistically significant difference ($p \leq 0.05$) between CA - DAD and CA - DAW was considered. The Wilcoxon test showed statistically significant difference between chronological age and dental age for both methods and both genders and the hypothesis was disclaimed.tabl. 12

Age group	Gender	n	Age	Median	P value за Wilcoxon signed-rank test
12,00-13,99	boys	32	CA	12.58	0,000
			DAD	14.4	
12,00-13,99	boys	32	CA	12.58	0,018
			DAW	13.59	
12,00-13,99	girls	67	CA	12.9	0,000
			DAD	13.7	
12,00-13,99	girls	67	CA	12.9	0,011
			DAW	12.88	

Tabl. 12 Comparison of mean values of CA, DAD, DAW for both genders in age group 12.00-13.99 years

The next step was to evaluate CA as overestimated or underestimated by DAD and DAW. The hypothesis of no difference between CA - DAD and CA- DAW was considered. With the Student's t-test, the hypothesis was disclaimed for DAD for both genders - for boys CA was overestimated by 0.66 -1.51 years, whereas the CA was overestimated by 0.84- 1.17 years for girls. DAW overestimated the CA for boys only by 0.07-0.81 year, whereas for girls the CA was overestimated only by 0.03-0.39 years. (tabl.13)

Age group	gender	N	Absolute difference	Mean Difference	SD	SE	95% Confidence Interval for Mean		t value	P value
							Lower Bound	Upper Bound		
12,00-13,99	boys	32	CA-DAD	-1,09	1,18	0,21	-1,51	-0,66	-5,21	0.000
			CA-DAW	-0,44	1,03	0,18	-0,81	-0,07	-2,43	0,021
	girls	67	CA-DAD	-1,00	0,68	0,08	- 1,17	-0,84	-12,11	0,000
			CA-DAW	0,21	0,74	0,09	-0,39	-0,03	-2,35	0,022

Tabl. 13 Comparison of the absolute difference of CA-DAD and CA-DAW for both genders in age group 12.00-13.99 years

Correlation between the difference of CA-DAW (r=0,11; p=0.44) and the deference of CA-DAD (r=0.04; p=0.72) with the gender was not found.

Results for Age group-14.00-15.99 years

The Kolmogorov-Smirnov test indicated an abnormal distribution of the data in this age group for both genders. Graphically the results are presented on Fig. 14 and fig. 15. The median of CA for boys is 14.78 years (IQR 14.21 - 15,59); DAD is 15.70 years (IQR 14.73 – 15.70); DAW – 14.34 years (IQR 13.78 – 14.34). The median of CA for girls is 14.55 years (IQR-14.25-15.14); DAD is 14.6 years (IQR 14.6-16.00); DAW- 13.84 years (IQR 13.84- 15.79)..

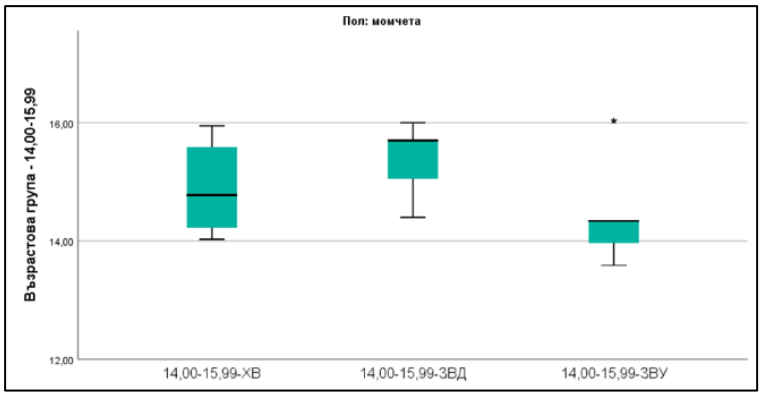


Fig. 14 box plot of CA; DAD; DAW in age group 14, 00-15, 99 for boys

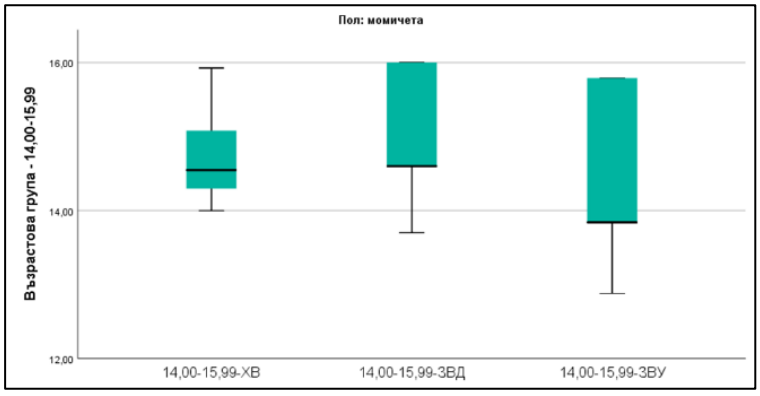


Fig. 15 box plot of CA; DAD; DAW in age group 14, 00-15, 99 for girls

The hypothesis of no statistically significant difference ($p \leq 0.05$) between CA and DAD and CA DAW was considered. The Wilcoxon signed-rank test showed statistically significant difference between chronological age and dental age for Demirjian method for both genders and Willems method for boys only. The difference (CA-DAW) was not statistically significant ($p=0.256$) for girls so the conclusion that in

this age group for female subjects the DAW estimate the CA correctly was done. Tabl. 14

Age group	Gender	N	Age	Median	P value за Wilcoxon signed-rank test
14,00-15,99	boys	20	CA	14.78	0,022
			DAD	15.7	
14,00-15,99	girls	31	CA	14.78	0,030
			DAW	14.34	
14,00-15,99	boys	20	CA	14.55	0,025
			DAD	14.6	
14,00-15,99	girls	31	CA	14.55	0,256
			DAW	13.84	

Tabl. 14 Comparison of mean values of CA, DAD, DAW for both genders in age group 14.00-15.99 years

The next step was to evaluate CA as overestimated or underestimated by DAD and DAW. The hypothesis of no difference between CA - DAD and CA- DAW was considered. With the Student's t-test, the hypothesis was disclaimed for DAD for both genders - for boys CA was overestimated by 0.18 -0.83 years, whereas the CA was overestimated by 0.04- 1.62 years for girls. DAW underestimated the CA for boys by 0.07-0.80 years. For girls the CA was underestimated also but the difference is not statistically significant ($p=0.25, \geq 0.05$) -tabl. 15

Age group	Gender	N	Absolute difference	Mean Difference	SD	SE	95% Confidence Interval for Mean		t value	P value
							Lower Bound	Upper Bound		
14,00-15,99	boys	20	CA-DAD	-0,50	0,70	0,16	-0,83	-0,18	-3,25	0,004
			CA-DAW	0,44	0,78	0,17	0,07	0,80	2,52	0,02
	girls	31	CA-DAD	-0,33	0,79	0,14	-0,62	-0,04	-2,32	0,02
			CA-DAW	0,21	1,01	0,18	-0,16	0,58	1,17	0,25

Tabl. 15 Comparison of the absolute difference of CA-DAD and CA-DAW for both genders in age group 14.00-15.99 years

Correlation between the difference of CA-DAW ($r=0.09$; $p=0.52$, $p \geq 0.05$) and the deference of CA-DAD ($r=0.12$; $p=0.41$, $p \geq 0.05$) with the gender was not found.

The both methods of age estimation-Demirjian [39] and Willems [160] were widely used in different populations. The results are controversial – DA overestimated or underestimated the CA. Studies of Maber et al (2006) and Liversidge et al. (2010) showed that both methods –Demirjian and Willems defined DA, which underestimated the CA. [99,96] The results of our study supported this thesis only for DA estimated with Willems method for boys in age group 14,00-15,99. The vast majority of studies performed by many researchers demonstrated results where the both

methods of DA estimation overestimated the CA.The results of our study showed that DAD overestimated CA in all four age groups for both genders. Whereas DAW overestimated the HA for both genders in all age groups except for boys aged 14,00-15,99 (underestimate the CA) and girls aged 7,00-9,99; 14,00-15,99 (DA coincident CA). Another author-Grover et al. (2011) [66] declared in his study that DAW is more accurate for females. Our study supported this statement but only for female subjects aged 7.00-9.99 years and 14.00-15.99 years. Researches like Ambarkova V. et al (2013) [8], Jeta Kelmendi et al. (2018) [80], Hegde S. et al. (2020) [72] compared the accuracy of DAW and DAD methods in their studies and declared superiority of DAW for estimation of CA.The results of our study absolutely supported this thesis. In age group 7,00-9,99 the Willems method overestimated the CA up to 6 months for boys and no difference between DA and CA was found for girls. In age group 10,00-11,99 in both genders Willems method exceeded the CA up to 12 months. In age group 12,00-13,99 for boys the overestimation of CA is up to 9 months, for girls up to 6 months. In the last age group 14,00-15,99 The Willems method underestimate the CA by up to 9 months, whereas no difference between DA and CA was found for girls. Acceptable ranges of age difference between estimated and chronological age in forensic anthropology of children until adolescence varies from (±0.5 year) as a stringent up to (±1.00 year) as a maximum acceptable difference. [54, 29]. The

results of our research showed difference (if present) between DAW and CA for all age groups for both genders by up to 1.00 year, which approve the accuracy of the DAW method for Bulgarian children aged 7-16 year old with mixed and permanent dentition.

Results by task 2:

We determined a mean CA for boys and girls at the maturation stage of the cervical vertebrae CVM II, CVM III and CVM IV , which outline the period of a year before the peak of puberty, the peak of puberty and a year after the pubertal spurt. The prepubertal period and the peak of pubertal growth are the most important for optimal orthodontic treatment of malocclusions with orthopedic correction by influencing and guiding growth processes in maxillofacial regeon.

Peak of pubertal growth in boys

The boys studied were in stage CVM II, CVM III or CVM IV divided into 12 age groups in 6 months

(0.5years) increments. (Fig. 16)

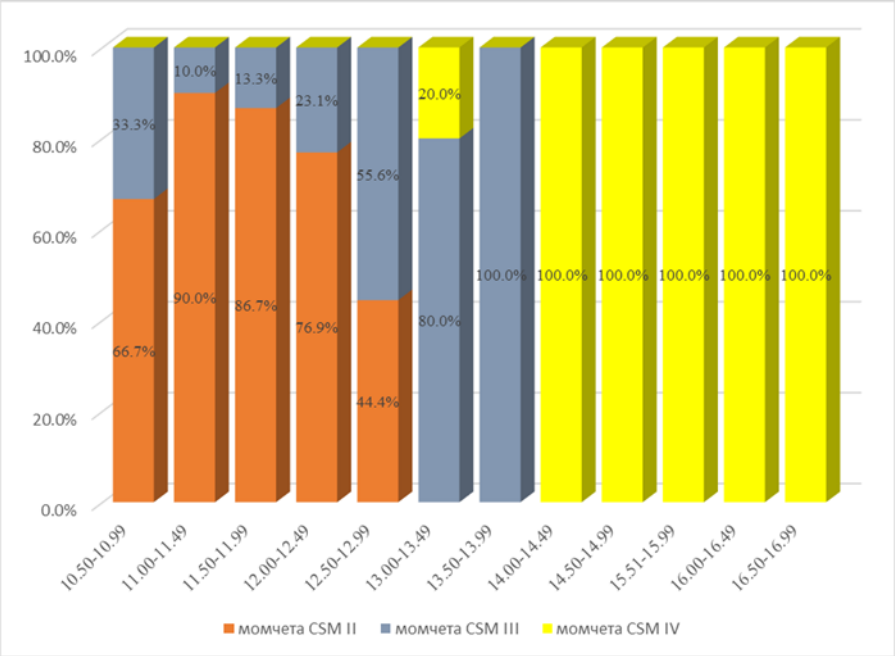


Figure 16: Distribution of the boys in stages of the cervical vertebrae maturation CVM II, CVM III, CVM IV in different age groups.

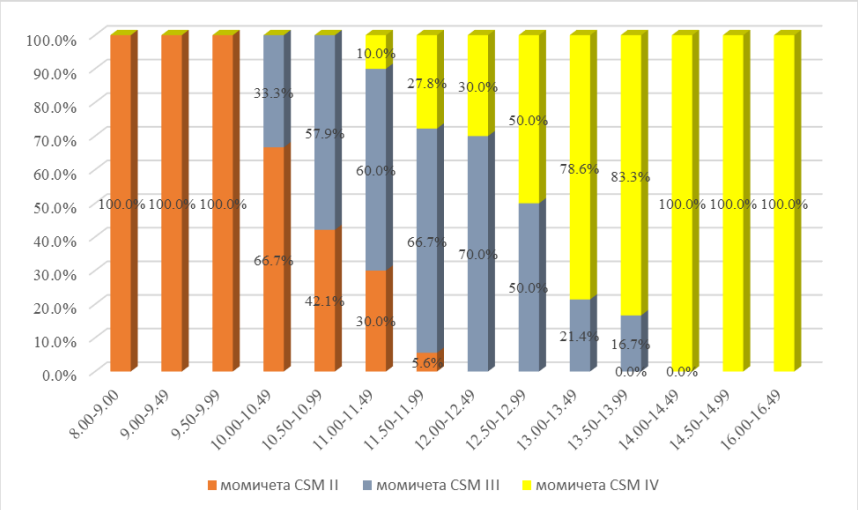
For the sample studied, the mean age of boys with CVM II was 11, 81 ± 0.53 years, stage CVM III - 12.52 ± 0.81 years, and the CVM IV maturity stage occurred at an average age in boys aged 14.82 ± 1.00 years. (table 16)

CVM	N	Mean	SD	SE	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
II	38	11.81	0.53	0.09	11.63	11.98	10.89	12.85
III	18	12.52	0.81	0.19	12.12	12.93	10.93	13.67
IV	13	14.82	1.00	0.28	14.21	15.42	13.45	16.53
Total	69	12.56	1.33	0.16	12.24	12.88	10.89	16.53
ANOVA: F = 86,75, p = 3,3665E-19 (p<0,05)								

Tabl. 16: Mean age in boys in the relevant stages of maturation.

Peak of pubertal growth in girls

All the girls who were in stage CVM II, CVM III or CVM IV were divided into 12 age groups every 6 months- (0.5 years) increments. (Fig. 17)



Fiura 17: Distribution of the girls in stages of the cervical vertebrae maturation CVM II, CVM III, CVM IV in different age groups

From the data obtained in the sample analysis, it was found that the mean age of the girls surveyed with CVM II was 10.22 ± 0.78 years. Stage of maturity CVM III, occurs at average age in girls 11.80 ± 0.81 years. Stage of maturity CVM IV occurs at an average age in girls 13.13 ± 0.94 years. (table 17)

CVM	N	Mean	SD	SE	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
II	26	10.22	0.78	0.15	9.91	10.54	8.54	11.89
III	56	11.80	0.81	0.11	11.58	12.02	10.14	13.56
IV	43	13.13	0.94	0.14	12.84	13.42	11.46	16.02
Total	125	11.93	1.35	0.12	11.69	12.17	8.54	16.02
ANOVA: F = 95,74 , p = 9,9903E-26 (p<0,05)								

Table 17: Mean age in girls in the relevant stages of maturation

Based on our research, with 95% accuracy, we can claim that the most appropriate age for girls to start orthodontic treatment with optimal orthopedic effect for correction of distal occlusion with retrognathic mandible, in which the growth management in stage CVM III is necessary is between 11.58 – 12.02 years.

Treatment of Class III with maxillary expansion and protraction with the best orthopedic effect should be carried out up to CVM II stage, which according to our research with 95% accuracy is to mean CA- 9.91-10.54 years for girls.

Based on our research, we can claim with 95% accuracy that the most appropriate age in boys for orthodontic treatment with optimal orthopedic effect in the correction of distal occlusion with retrognathic mandible, in which the growth guidance is necessary should begin in stage CVM III with mean CA- 12.12 -12. 93 years.

Treatment of Class III with maxillary expansion and protraction with the best orthopedic effect should be carried out up to CVM II stage , which according to our research with 95% accuracy is to mean CA - 11.63-11.98 years for boys.

Results by task 3

BOYS:

At the stage of skeletal age CVM II in boys for teeth 33, 35, 37 and 23 predominated stage of mineralization was G of Demirjian. For tooth 38- stage D -63.6% respectively. At the stage CVM III in boys for tooth 23 and 33 the predominant stage was stage H of Demirjian. For teeth 35 and 37 prevailed stage was still G of Demiejian. For tooth 38- stage D- 68.8%, respectively. At CVM IV for boys the teeth 23 and 33 were in stage H determined in 100% of the male studied, and in 70% of them tooth 35 was also in stage H. For tooth 38 stage D was defined in 69.2% (Fig. 18, 19, 20, 21, 22)

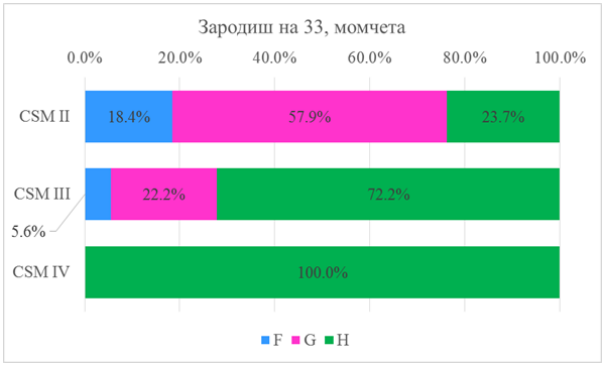


Figure 18: Calcification stages of 33 in boys in CVM II, CVM III, CVM IV

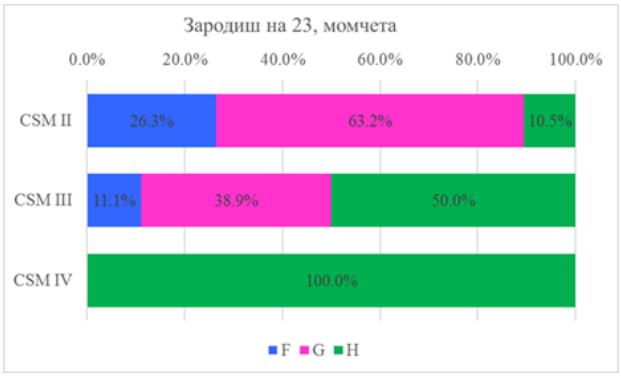


Figure 19: Calcification stages of 23 in boys in CVM II, CVM III, CVM IV

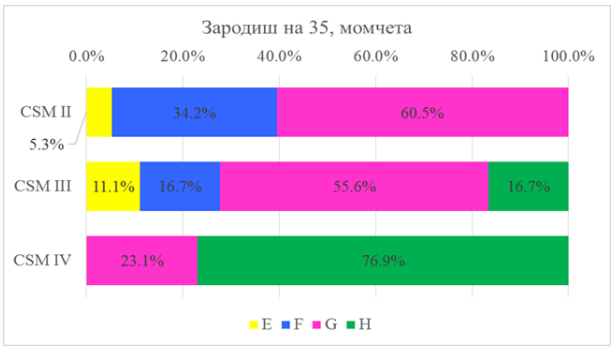


Figure 20: Calcification stages of 35 in boys in CVM II, CVM III, CVM IV

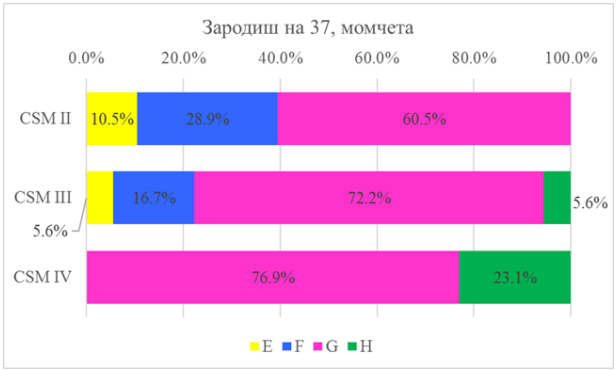


Figure 21: Calcification stages of 37 in boys in CVM II, CVM III, CVM IV

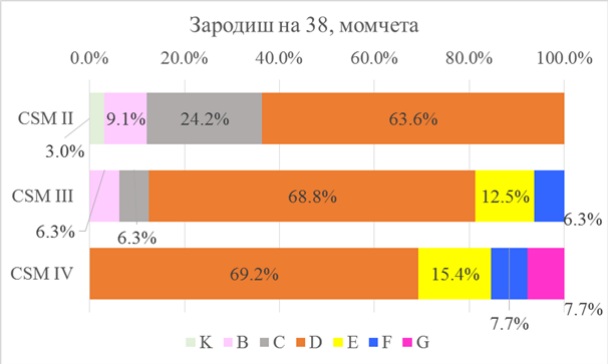


Figure 22: Calcification stages of 38 in boys in CVM II, CVM III, CVM IV

GIRLS:

At the stage of CVM II in girls for tooth 33 and 23 predominated stage of mineralization was G of Demirjian. For teeth 35 and 37 was stage F. For tooth 38- stage C, respectively. At the stage CVM III in girls for tooth 33 it was stage H defined in 85.7%. Teeth 23, 35 and 37 were still with predominated stage G. Respectively, for tooth 38- stage D. At the skeletal age CVM IV in girls for tooth 33 - stage H was determined in 100 % of the female children studied, and in 93% of them tooth 23 was also in stage H. Tooth 35 was in stage H at 51.2% of the cases. Tooth 37 was still in stage G at 95.3%. For tooth 38- stage D 75% respectively. (Fig. 23, 24, 25, 26, 27)

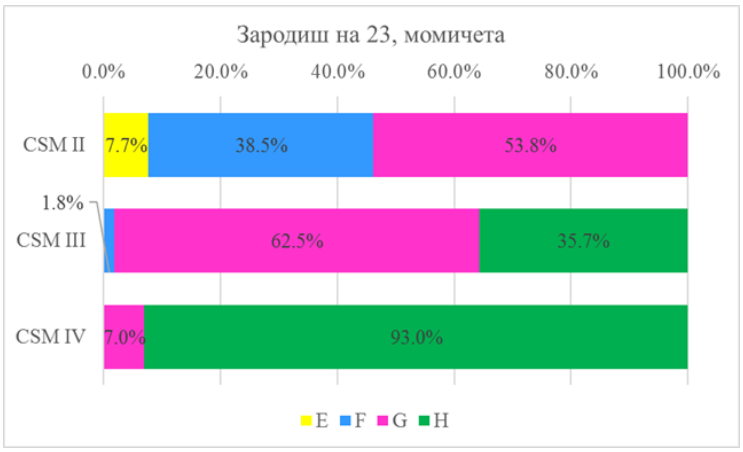


Figure 23 : Calcification stages of 23 in girls in CVM II, CVM III, CVM IV

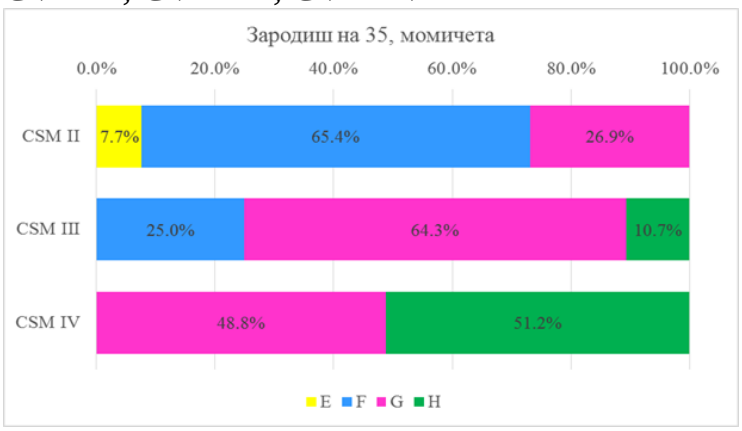


Figure 24 : Calcification stages of 35 in girls in CVM II, CVM III, CVM IV

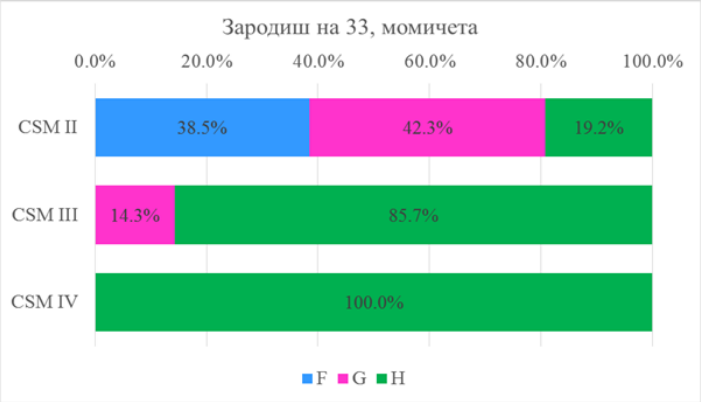


Figure 25 : Calcification stages of 33 in girls in CVM II, CVM III, CVM IV

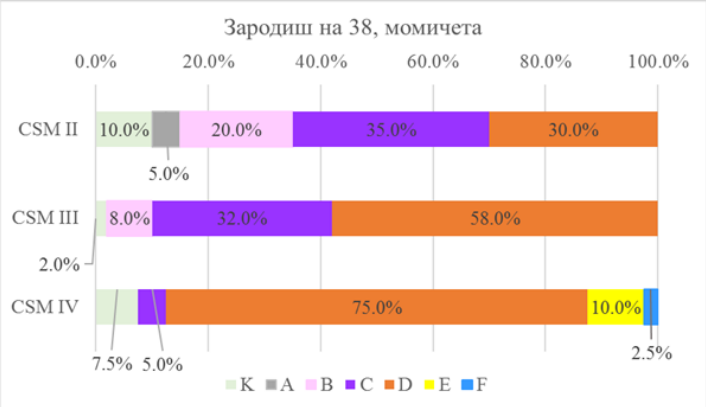


Figure 26 : Calcification stages of 38 in girls in CVM II, CVM III, CVM IV

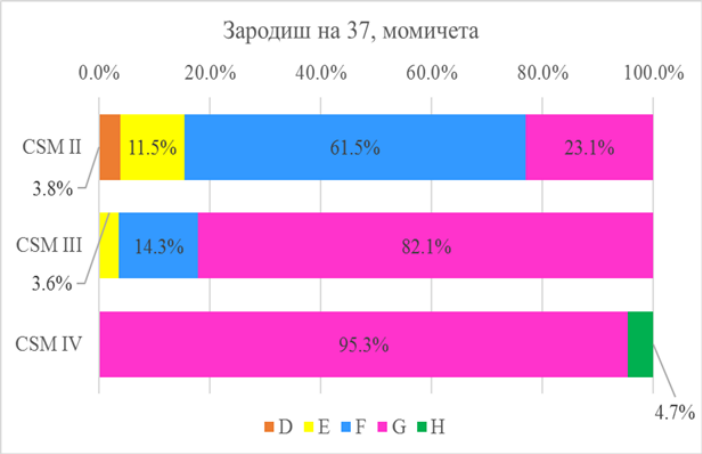


Figure 27 : Calcification stages of 37 in girls in CVM II, CVM III, CVM IV

Results and discussion on task 4:

The strongest correlation in girls was found between the mineralization stage of maxillary left canine (r =0.75), followed by mandibular left premolar (r=0.66), mandibular left canine (r=0.64) and mandibular second molar (r=0.61). Moderate is a correlation between the stage of mineralization of the mandibular left third molar-38 and Cervical vertebrae maturational stages of Baccetti et al.(r =0.45) (tabl.18)

	Stage of mineralization of tooth 33	Stage of mineralization of tooth 35	Stage of mineralization of tooth 37	Stage of mineralization of tooth 38	Stage of mineralization of tooth 23
r (Spearmen)	0,637**	0,659**	0,609**	0,448**	0,747**
P	0.000	0.000	0.000	0.000	0.000
N	125	125	125	110	125

Table 18: Correlation in girls between mineralisation stages of teeth 33, 35, 37, 38, 23 and maturation stages CVM II, CVM III, CVM IV.

The most commonly defined stages of mineralization of the teeth with the highest correlation are 23 (r = 0.75) , 35 (r = 0.66) , 33 (r = 0.64) and 37 (r=0.61) were compared with the stages of maturation CVM II, CVM III, CVM IV. (Table 19)

GIRLS					
	33	35	37	38	23
CVM II	G	F	F	C	G
CVM III	H	G	G	D	G
CVM IV	H	H	G	D	H

Table 19: Most Commonly Determined Degree of Mineralization of Dental Germs in Maturation Stages CVM II, CVM III, CVM IV in Girls

In boys, a significant correlation was found between the mineralisation stage of the maxillary left canine (r =0.64), followed by mandibular left canine (r =0.61) and mandibular second premolar (r =0.54). Moderate is a correlation between the mineralization stage of the mandibular left second molar-37 (r =0.41) and the mandibular third molar 38 (r =0.48) with the Maturation stages of Baccetti et al.(table 20)

	Stage of mineralization of tooth 33	Stage of mineralization of tooth 35	Stage of mineralization of tooth 37	Stage of mineralization of tooth 38	Stage of mineralization of tooth 23
r (Spearmen)	0,605**	0,544**	0,407**	0,481**	0,643**
P	0.000	0.000	0.001	0.000	0.000
N	69	69	69	62	69

Table 20: Correlation in boys between tooth mineralisation stage 33, 35, 37, 38, 23 and maturation stages CVM II, CVM III, CVM IV.

The most commonly defined stages of mineralization of the teeth with the highest correlation - maxillary left canine (r =0.64), followed by mandibular left canine (r =0.61) and mandibular second premolar (r =0.54) in boys, were compared with the stages of maturation CVM II, CVM III, CVM IV. (table 21)

	33	35	37	38	23
CVM II	G	G	G	D	G
CVM III	H	G	G	D	H
CVM IV	H	H	G	D	H

Table 21: Most Commonly Determined Degree of Mineralization of Dental Germs in Maturation Stages CVM II, CVM III, CVM IV in Boys

In summary of the results of the tasks assigned in the dissertation , we prepared a model for determining the puberty period of growth on orthopanthogram, by assessing individual indicators of growth and development in girls (scheme 1) and in boys (scheme 2).

	Maturational stage of Baccetti	Chronological age (years)	Demirjian dental germ minaralization stage			
			33	23	35	37
GIRLS	CVM II	9, 91 – 10, 54	G	G	F	F
	CVM III	11, 58- 12, 02	H	G	G	G
	CVM IV	12, 84 –13,42	H	H	H	G

Scheme 1: Individual indicators for determining the pubertal growth period in girls

	Maturational stage of Baccetti	Chronological age (years)	Demirjian dental germ minaralization stage		
			33	23	35
BOYS	CVM II	11, 63 – 11, 98	G	G	G
	CVM III	12, 12 - 12, 93	H	H	G
	CVM IV	14, 21 –15, 42	H	H	H

Scheme 2: Individual indicators for determining pubertal growth period in boys

V. CONCLUSIONS

Conclusions on Task 1

1. Demirjian's method overestimates the CA in all age groups in both genders. For girls in the group of 10.00-11.99 year old the difference being almost 2 years above the actual CA.

2. Willems' method also showed overestimation of chronological age, but within smaller limits - less than 12 months, for all age groups in both genders except girls of age group 7.00-9.99 year old and from 14.00-15.99 year old, in which the DAW coincident with CA.

3. Willems method showed underestimation of CA in the group of 14.00-15.99 year old boys, with an ranged from 0.07 to 0.80 years .

4. In the group of 10.00-11.99 year old the difference between the dental age determined by the Demirjian method and chronological age is in correlation with gender. The method determines the DAD more inaccurately in girls than in boys.

5. The DA calculated by the Willems method in girls is close to CA, while the Demirjian method overestimates by 0.35 to 0.9 years in the age group 7.00-9.99 years.

6. Willems method, overestimating the CA in the group of 10.00-11.99 year old boys in the range from 0.31 and 0.94 years, and for girls - from 0.74 to 1.06 years, while in the group of 12.00-13.99 year old the overestimation is significantly less and was in the

range of 0.07 to 0.81 years for boys and for girls from 0.03 to 0.39 years .

Conclusions on Task 2

1. Analysis of cervical vertebrae maturation showed that in the 11-year-old's group boys, the number of people who had been in CVM II stage- approximately 1 year before puberty peak is 76 % and only 10% are at the peak of puberty. At 12 years of age, pre-peak puberty boys at stage CVM II were 58% and only 33% were at the peak of growth.

2. The peak of pubertal growth in boys occurred around 13 years at stage CVM III for 75% of the children studied. At the age of 14 - 73% of boys were in CVM IV - (1 year after the peak of puberty). Boys aged 15 and 16 years were predominantly in stage- V, but there is still not a small percentage of children in the previous stage.

3. Analysis of the maturity of the cervical vertebrae in girls showed that at 9 years of age they enter the puberty period of growth in 57% with stage CVM II . In the group of 10 year old 50% of girls reached the peak of puberty-stage CVM III, while the rest were still in pre-peak period- CVM II - 46%.

4. The highest proportion of girls at puberty peak were those 11 and 12 years old – 64% and 62%, respectively.

5. The number of girls in stage CVM IV (one year after puberty) changed from 21% in 11 years, through 38% in 12 years and in the group of 13 year old girls has already reached 47%.

6. The mean chronological age of boys with CVM II was 11, 81 ± 0.53 years. Stage CVM III was established in boys at 12.52 ± 0.81 years, and stage of maturity CVM IV occurred at an average age in boys at 14.82 ± 1.00years.

7. The mean chronological age of girls with CVM II was 10.22 ± 0.78 years. CVM III stage of maturity occurs at an average age in girls 11.80 ± 0.81 years, and the CVM IV maturity stage occurs at 13.13 ± 0.94 years.

8. In the 17.00-year-old girls group, 100% of the children studied were in the CVM VI maturation stage, while in 17-year-old boys, not a single child in stage CVM VI was reported.

Conclusions on Task 3

1. In stage CVM II, the mineralization and degree of maturation of the mandible left canin indicates that the tooth apex is not closed in 76.3% of boys and 80.8% of girls, while during the peak of puberty- CVM III the root apex is completely closed (Stage H of Demirjian) in 72.2% of boys and 85.7% of girls.

2. At the stage of maturation of the cervical vertebrae CVM II, maxillary canine has an open apex in 89.5% of boys and 100% of girls, while at the peak of puberty- stage CVM III in 50% of the boys studied, maxillary canine has closed apex, but in the remaining 50% the root development continues not to be completed. In 64.3% of girls, tooth apex 23 remains unclosed at the peak of puberty.

3. In boys in the CVM II maturation stage, teeth 35 and 37 had an unclosed apex in 100% of the children studied and this was maintained at the peak of puberty- stage CVM III in 83.3% of the boys studied for tooth 35 and in 94.4% of boys for tooth 37. In girls for teeth 35 and 37 in the CVM II stage, all apexes are opened with stage F of Demirjian, while at the peak of growth the apexes are still open with stage G of Demirjian.

Conclusions on Task 4

1. A significant correlation in boys was found between the stages of maturity of the cervical vertebrae CVM II, CVM III and CVM IV by the Baccetti et al. method and the mineralization stage of the maxillary left canine (r =0.64), followed by mandible left canine (r =0.61) and mandible second PM (r =0.54).

2. The strongest correlation in girls was found between the mineralization stage of maxillary left canine (r =0.75), followed by the mandible left premolar (r=0.66), the mandible left canine (r=0.64) and the mandible second molar (r=0.61) and the maturity stages of the cervical vertebrae CVM II, CVM III and CVM IV of Baccetti et al. method.

3. In all female children studied (100%) at the stage of maturation CVM II and chronological age 9.91-10.54 , teeth 23, 35 and 37 had incomplete root development, with 35 and 37 in stage F in the majority of cases, and tooth 23 and 33 in stage-G.

4. The studied females in the stage CVM III and the average CA 11.58-12.02 years showed that in 85.7% of girls tooth 33 had completed root development, while 23, 35 and 37 teeth remained with open apex in the majority of cases. In the girls studied in CVM IV 93% of tooth 23 is in stage-H and at 100% tooth 33 is in stage H-completed root development of the two canines at a year post pubertal peak of development.

5. The studied boys in the stage of bone maturation CVM II have an average CA of 11.63-11.98 years and the maxillary and mandible canine have incomplete root development and open apex with a predominant stage of mineralization-G. In 100% of the boys studied in the stage of bone maturation CVM II tooth 35 has the incomplete root development with a predominant stage of mineralization - G.

6. In boys at the stage of bone maturation CVM III, the mean CA is 12.12-12.93 years. With a closed apex is mandible canine-33 in 72% and 23 tooth in 50% of the boys studied .With an unclosed apex remains tooth 35 in 55.6% of the male children studied.

7. In the boys studied in CVM IV, 100% tooth 23 is in stage-H and 100% tooth 33 is in stage H-completed root development of both canines at a year post peak period of the puberty.Tooth 35- 76.9% was in stage H.

VI. INFERENCES

1. Willems et al.(2001) method for assessing DA in Bulgarian children in mixed and permanent dentition aged 7-16 years can be considered an accurate and reliable method, as it did not show a difference with the actual age by more than 12 months in either boys or girls, in all age groups.

2. Demirjian et al. (1973) method for assessing DA in Bulgarian children in mixed and permanent dentition aged 7-16 years cannot be considered an accurate and reliable method, as it showed a significant difference with the actual age of over 12 months in most age groups in both sexes

3. The most appropriate age for orthodontic treatment with optimal orthopedic effect in the correction of distal occlusion with retrognatic mandible, in which the growth menagment is necessary should start at stage CVM III with 95 % accuracy in the mean age of 12.12-12.93 years for boys and 11.58 – 12.02 years for girls.

4. Treatment of Class III with maxillary expansion and protraction with optimal orthopedic effect should finish at stage CVM II with 95 % accuracy in the mean age of 11.63-11.98 years for boys and 9.91 – 10.54 years for girls.

5. Girls matured earlier -average CA of the onset of puberty development was 9.91-10.54 years, compared to that of boys- average 11.63-11.98 years of puberty.

6. One year before the puberty peak in boys (CVM II, the mean CA was 11.63-11.98years). Mineralisation and tooth maturation stages of teeth 23, 33, 35 and 37 were in Stages G of Demirjian.

7. At the puberty peak in boys (CVM III, the average CA was 12.12-12.93 yeras) the apexes of teeth 23 and 33 are closed and passed into stages H of Demirjian.

8. One year before puberty peak in girls CVM II , the average CA was 9.91-10.54 years). Mineralization stage of teeth 23 and 33 is stage G of Demirjian, while teeth 35 and 37 are in stage F of Demirjian .

9. At the peak of puberty in girls (CVM III , the average CA was 11.58-12.02 years), the apex is closed only on tooth 33, while the teeth- 23, 35 and 37 were in stage G of Demirjian.

10. The age, gender and degree of mineralization of certain teeth in combination are reliable indicators of skeletal maturation determined by the OPG.

VII. Contributions

Contribution with an original character for the country :

For the first time in Bulgaria, a model is prepared for determining the puberty period of growth on orthopanthogram by assessing individual indicators of growth and development.

Contributions of a scientific and theoretical nature:

1. An assessment of the dental age by the Demirjian method and the Willems method in Bulgarian children aged 7-16 years was studied.

2. The accuracy of each method in determining the dental age relative to chronological ages and between both methods has been examined.

3. The correlation between the accuracy of the methods of assessing dental age by Demirjian and Willems and gender has been investigated.

4. The average chronological age was established in girls and boys prior to the peak of puberty development at stage CVM II, at the peak of puberty at stage CVM III and after the peak of puberty in stage CVM VI by the Baccetti method

5. The stages of mineralization of teeth 23, 33, 35, 37, 38 during the stages of skeletal age - CVM II, CVM III and CVM IV have been established.

6. The correlation relationships between the stages of mineralization of teeth 23, 33, 35, 37, 38 and the stages of bone age CVM II, CVM III and CVM IV have been studied.

Contributions of a scientifically and practically applied nature:

1. We recommend Willems method for assessing dental age in Bulgarian children aged 7-16 years.

2. We established the average chronological age of girls and boys until reaching it, the most effective treatment of Class III with maxillary expansion and upper jaw protraction can be done.

3. We established the mean chronological age of girls and boys at the time of reaching it the start of treatment of distal bite with retrognathic mandible should begin.

4. We determined the stages of mineralization of teeth 23, 33, 35, 37, 38 during the stages of skeletal age CVM II, CVM III and CVM IV and the relationship between them.

5. We have prepared a model for determining the puberty period of growth on orthopanthograpam, by assessing the degree of mineralization of different teeth in combination with chronological age and gender.

VII. Publications related to the dissertation

Articles :

Atanasova I., Dental age estimation using the Willems method in children with mixed and permanent dentition: a digital orthopantomographic study, Journal of the Union of Scientists- Varna. Medicine and Ecology Series 2021 vol. 26, №2

ISSN 1310-6031(Print); ISSN 2603-4565(Online)

Atanasova I., Correlation between maxillary canine calcification stages and skeletal maturation (skeletal age) Journal of the Union of Scientists- Varna. Medicine and Ecology Series 2021 vol. 26, №1

ISSN 1310-6031(Print); ISSN 2603-4565(Online)

Atanasova, I. Evaluation of skeletal maturity using mandibular canine calcification stages. Abstract book, IV congress of Balkan association of orthodontic specialists, 10-13 September 2020, Sofia.

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PARTICIPATION IN SCIENTIFIC FORUMS:

1. IV congress of Balkan association of Orthodontic specialists, 10-13 September 2020, Sofia

Atanasova, I. Evaluation of skeletal maturity using mandibular canine calcification stages-poster presentation

2. Scientific forums- Union of Scientists in Bulgaria-Varna October 2021.

Atanasova, I. "Dental age estimation using Willems method in children with mixed and permanent dentition: A digital orthopantomographic study"