applied sciences

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Cephalometric Changes Following Maxillary Expansion with Ni-Ti Leaf Springs Palatal Expander and Rapid Maxillary Expander: A Retrospective Study

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Abstract: Background: The aim of this study is to evaluate and compare, through bidimensional cephalometry, skeletal and dental changes obtained from a rapid maxillary expander (RME) and a Ni-Ti leaf spring expander (Leaf) and compare them with an untreated control group. Methods: Records consisted of lateral cephalograms obtained before and after maxillary expansion of patients that underwent orthodontic treatment at the Department of Biomedical, Surgical and Dental Sciences. The Leaf expander group consisted of 9 males (mean age = 7.5 ± 0.9 years old) and 11 females (mean age = 8.2 ± 0.6 years old). The RME group of the present study was composed of 11 males (mean age = 7.8 ± 0.6 years old) and 12 females (mean age = 8.1 ± 0.5 years old). Digital cephalograms were traced using Dolphin Imaging software v.11.1 (Dolphin Imaging and Management Solutions; Los Angeles, CA, USA), which calculated all reported measurements. Each subject was assigned a random identification number, and the examiner was blinded to the subject when measuring. The difference between the two experimental times in all groups was evaluated using the Student's *t*-test for dependent variables. The difference between the two evaluation times in each group for all the variables was used to perform a one-way ANOVA test between the three groups. Results: No statistically significant difference was noted, apart from the angle between the upper incisor and the SN and PP planes, which showed an average decrease of 3.25 and 2.55, respectively, and the angle between the lower incisors and the mandibular plane, which showed an average increase of 2.85 degrees. The one-way ANOVA showed no statistically significant difference between the three groups. Conclusions: It appears that the leaf expander and the RME present similar effects such as dental and skeletal changes.

Keywords: maxillary hypoplasia; maxillary expansion; lateral cephalogram; cephalometry

Citation: Lanteri, V.; Abate, A.; Cavagnetto, D.; Ugolini, A.; Gaffuri, F.; Gianolio, A.; Maspero, C. Cephalometric Changes Following Maxillary Expansion with Ni-Ti Leaf Springs Palatal Expander and Rapid Maxillary Expander: A Retrospective Study. *Appl. Sci.* **2021**, *11*, 5748. https://doi.org/10.3390/app11125748

Academic Editors: Gianrico Spagnuolo and Gabi Chaushu

Received: 25 May 2021
Accepted: 17 June 2021
Published: 21 June 2021

1. Introduction

Orthopedic maxillary expansion can be achieved using different fixed appliances that use deciduous or permanent teeth or miniscrews for support to transmit a lateral force to the two palatal processes of the maxillary bone, producing the splitting of the midpalatal suture and a certain amount of widening of the maxillary sutures [1]. The force is generated by a transversal screw that delivers a variable amount of force depending on several factors such as the activation protocol, its relative position in the palatal vault and its type of support. Palatal expansion allows decreasing nasal resistances and enlarging the upper airways, therefore improving breathing [2,3].

It also allows correcting crossbite, thus preventing the worsening of craniofacial asymmetries during growth [4,5].

Rapid maxillary expansion is a common activation protocol of the median screw that allows correcting maxillary hypoplasia and posterior crossbite in 1/3 weeks by activating the median screw of the appliance twice a day (total activation = 0.4–0.5 mm) [6]. The other commonly used expansion protocol is slow maxillary expansion, which requires less frequent activation of the median screw for a longer period of time. Despite the different kinds of protocols, both of the maxillary expanders have similar orthopedic effects in growing subjects [7].

A Ni-Ti leaf spring palatal expander (leaf expander) has a small size body and is similar to a conventional Hyrax expander [8]. The maxillary expansion is promoted by calibrated and continuous forces. In particular, the constant lateral force of 450 g delivered by the Ni-Ti leaf spring allows for a 6 or 9 mm maximum expansion. The clinician reactivates the device once a month with 10 quarter turns (1 mm of activation) until the achievement of the needed expansion. The advantage of the presented appliance leads to no required compliance from patients' parents; moreover, it is less painful compared to conventional expanders and provides a similar amount of expansion [9–13]. Moreover, leaf expander treatment appeared effective in increasing upper airway and maxillary sinus volume in patients with maxillary hypoplasia, demonstrating no difference from a conventional Hyrax RME [9].

The aim of this study is to evaluate and compare, through bidimensional cephalometry performed on lateral radiographs, sagittal and vertical skeletal and dental changes obtained from an RME and a leaf expander anchored to deciduous teeth and compare them with an untreated control group.

2. Materials and Methods

2.1. Study Design and Type of Participants

A retrospective study on changes of the skeletal and dental structures before and after maxillary expansion obtained by an RME and a Leaf expander was performed using lateral radiographs of subjects treated at Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico Milan between January 2019 and August 2020.

The Ethical Committee of the Fondazione IRCCS Ca'Granda, Ospedale Maggiore, Milan—Italy (Protocol No.: 573/15) approved the present study protocol. Patients' parents or their guardians signed an informed consent form, allowing us to use diagnostic records for research motivations.

Records consisted of lateral cephalograms obtained before and after maxillary expansion of patients that underwent orthodontic treatment at the Department of Biomedical, Surgical and Dental Sciences.

The inclusion criteria for the samples of this observational retrospective study were as follows: good state of health; subjects with maxillary transverse deficiency (MTD) with unilateral/bilateral posterior crossbite; mixed dentition; age between 7 and 9 years old; an RME and a Leaf expander anchored on the upper deciduous second molars; patients before the pubertal peak of growth (CVM 1–3) [14].

Exclusion criteria were: lack of postexpansion lateral cephalograms; poor radiographic quality; patients with previous orthodontic treatment, hypodontia in any quadrant excluding third molars; inadequate oral hygiene; craniofacial syndromes or cleft lip or palate.

The lateral cephalometric head films were taken as follows: the first was taken no more than two months before the cementation, and the second was taken immediately after removing the appliance, approximately in a time period between 9 and 12 months from the beginning of the treatment. The RME requires a specific period of time from the last activation to stabilize orthopedic results. The overall mean duration, including active treatment and the retention phase, lasts 10 months according to the literature [15] and approximately 9 months with the Leaf expander [9].

According to Baccetti et al. [16], the prepubertal age was chosen because it is the most indicated period to resolve maxillary hypoplasia. Greater and more stable skeletal changes occur in subjects undergoing maxillary expansion during this particular stage of development.

A total of 23 patients treated with the rapid maxillary Hyrax expander and 20 subjects treated with the Leaf expander met the inclusion criteria and were included in the test groups.

The Leaf expander group consisted of 9 males (mean age = 7.5 $\pm$ 0.9 years old) and 11 females (mean age = 8.2 $\pm$ 0.6 years old). The mean distance between the two lateral radiographs was 10.2 months.

The RME group of the present study was composed of 11 males (mean age = 7.8 $\pm$ 0.6 years old) and 12 females (mean age = 8.1 $\pm$ 0.5 years old). The mean distance between the two lateral radiographs was 10.8 months.

The control group consisted of 19 untreated subjects: 10 males (mean age = 7.4 $\pm$ 0.8 years old) and 9 females (mean age = 7.7 $\pm$ 0.9 years old) who met the inclusion criteria. These patients did not receive any orthodontic treatment before taking the first lateral radiographs, and not even in the period between the first and the second. The two lateral cephalograms were taken as control to assess skeletal growth, as the parents refused a maxillary expansion treatment. The mean distance between them was 11 months.

2.2. Maxillary Expansion Protocol

Both the Leaf expander and the RME were cemented on the upper deciduous second molars [17].

The leaf expander is similar to a conventional rapid palatal expander, but instead of being formed by a jackscrew medially, it consists of a double Ni-Ti leaf spring.

By activating the leaf spring, it delivers a 6 mm maximum expansion and generates a continuous force of 450 g.

The maxillary expansion protocol for the Leaf expander was as follows: at the moment of cementation, the device was preactivated by the laboratory to deliver 3 mm of expansion, after which reactivation (compressing the leaf springs) was performed by the clinician in the office at subsequent appointments, giving 10 quarter turns (one quarter turn corresponds to 0.1 mm) of activation of the screw per month until the expansion was achieved [18].

The maxillary expansion protocol for patients that underwent RME consisted of performing a turn of the screw twice a day for the first 7 days. Parents' patients or their guardians were instructed to activate the appliance directly at home. The orthodontist then reevaluated the patients after 1 week and decided to stop or continue to activate the appliance until the completed expansion.

In both cases, when needed, the Hyrax screw and the Ni-Ti spring were blocked when first molar overcorrection was obtained, i.e., when the palatal cusps of the maxillary first permanent molars occluded on the edge of the lingual side of buccal cusps of the lower first permanent molars, as described by Caprioglio et al. [19].

2.3. Cephalometric Analysis

Lateral cephalograms of the skulls for each patient were taken with the same machine, Orthophos XG (Sirona Group, Bensheim, Germany), with a fixed-focus sensor distance (150 cm), at the Dental and Maxillofacial Department of the Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy.

Digital cephalograms were traced by a single operator (A.A.) using Dolphin Imaging software v.11.1 (Dolphin Imaging and Management Solutions; Los Angeles, CA, USA), which calculated all reported measurements.

Each subject was assigned a random identification number, and the observer was blinded to the subject when tracing. Accuracy of the landmark location of the anatomic outlines was tested on 20 randomly selected lateral radiographs by a second senior clinician

(D.C.) and then retraced by the first operator after 15 days in order to evaluate intra- and interoperator variability.

The following measurements were taken into consideration [20]: SNA; SNB; ANB; S-N ∧ Go-Gn; SNP-SNA ∧ Go-Gn; S-N ∧ SNP-SNA; NŜAr; SÂrGo; ArĜoGn; U1 ∧ S-N; U1 ∧ SNP-SNA; L1 ∧ Go-Me; U1 ∧ L1

All the cephalometric points are reported in Figure 1, and the cephalometric measurements analyzed are described in Table 1.

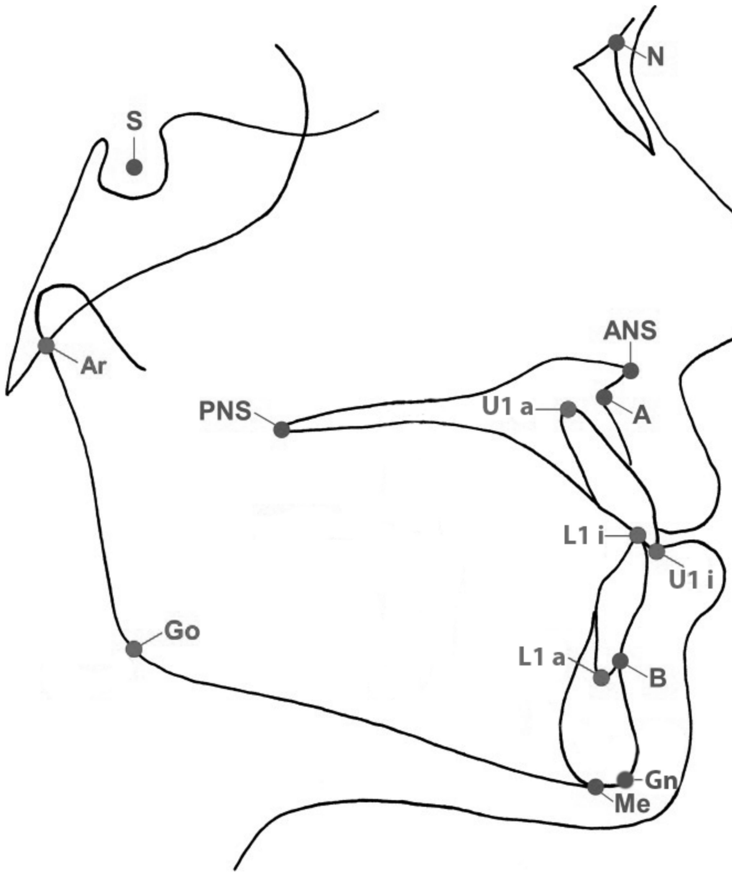

Figure 1. Description of the cephalometric points included in this study. S = Sella; N = nasion; ANS = anterior nasal spine; PNS = posterior nasal spine; A = Point A (most concave point of the anterior maxilla); B = Point B (most concave point of the mandibular symphysis); U1 a = upper incisor root apex; L1 apex = lower incisor root apex; U1 i = incisal edge of the upper incisor; L1 i = incisal edge of the lower incisor; Me = Menton (most inferior point of mandibular symphysis); Gn = gnathion (midpoint between the pogonion point and Me); Ar = (junction between the inferior surface of the cranial base and the posterior border of the ascending rami of the mandible.

2.4. Statistical Analysis

G*Power software (version 3.1.9.4, Franz Faul, Universitat Kiel, Kiel, Germany) was used to perform the sample size calculation based on the mean value with the corresponding standard deviation of the molar maxillary expansion (MME) obtained with the RME by Cerruto et al. [18]. Sample size was computed to obtain a statistical power of the study greater than 0.80 at an alpha of 0.05. Based on these parameters, the sample size required was 18 patients.

SPSS ® 23 for Windows (IBM, Sommers, NY, USA) was used for the statistical analysis.

After the normality assessment of data using the Shapiro–Wilk test, the difference at T0 between the three selected samples in the present study was evaluated using a one-way ANOVA test.

The difference between the two experimental times in all groups was evaluated using the Student's *t*-test for dependent variables. The difference between the two evaluation times in each group for all the variables was used to perform a one-way ANOVA test between the three groups. Intraclass correlation coefficients (ICCs) were computed for the variables taken into consideration. Intraclass correlation coefficients for the skeletal measures were greater than 0.94. Linear measurement errors averaged 0.3 mm (standard deviation (SD) = 0.4 mm) and angular measurements averaged 0.4 (SD = 0.3). A value of $p < 0.05$ was considered significant.

Table 1. Description of the considered cephalometric indexes.

	Description
SNA	Craniomaxillary angle: sagittal position of the maxilla
SNB	Craniomandibular angle: sagittal position of the mandible
ANB	Relative anteroposterior position between the maxilla and the mandible
S-N ∧ Go-Gn	Craniomandibular divergence
SNP-SNA ∧ Go-Gn	Maxillomandibular divergence
S-N ∧ SNP-SNA	Craniomaxillary divergence
NŜAr	Cranial base angle
SÂrGo	Articular angle
ArĜoGn	Gonion angle
U1 ∧ S-N	Upper incisor to the cranial plane
U1 ∧ SNP-SNA	Upper incisor to the palatal plane
L1 ∧ Go-Me	Lower incisor to the mandibular plane
U1 ∧ L1	Interincisal Angle

3. Results

No statistically significant difference was found at T0 between the three groups, confirming the homogeneity of the three selected samples.

Descriptive statistics and statistical assessments of cephalometric variables in each treatment group before and after therapy are shown in Table 2. No statistically significant difference was noted, apart from the angle between the upper incisor and the SN and PP planes, which showed an average decrease of 3.25 and 2.55, respectively, and the angle between the lower incisors and the mandibular plane, which showed an average increase of 2.85 degrees.

The one-way ANOVA (reported in Table 3) showed no statistically significant difference between the three groups.

The values for the intra- and interobserver agreement (ICC) for the cephalometric measurements (reported in Table 4) were 0.970 (95% CI: 1.000–0.934; $p < 0.001$) and 0.956 (95% CI: 0.985–0.927; $p < 0.001$). Overall, the method error was considered negligible.

Table 2. Cephalometric measurements of the three groups reported at T0 and independent *t*-test analyses at baseline.

	RME		SME		Control		
	T0		T0		T0		Independent *t*-Test
	Mean	SD	Mean	SD	Mean	SD	*p*-Value
SNA	83.52	4.99	79.75	4.38	81.73	6.01	0.14
SNB	78.48	6.46	76.55	3.87	78.73	4.75	0.84
ANB	6.04	2.94	3.75	2.17	3.27	2.18	0.47
S-N ∧ Go-Gn	34.96	6.06	34.85	6.23	34.20	5.26	0.52

Table 2. *Cont.*

	RME		SME		Control		
	T0		T0		T0		Independent *t*-Test
	Mean	SD	Mean	SD	Mean	SD	*p*-Value
SNP-SNA ∧ Go-Gn	27.74	4.74	26.95	7.03	25.80	5.83	0.28
S-N ∧ SNP-SNA	7.61	2.84	8.25	5.29	7.07	5.75	0.32
NŜAr	124.00	5.66	123.20	7.45	121.07	7.90	0.33
SÂrGo	139.91	6.37	143.80	10.94	144.93	9.40	0.65
ArĜoGn	132.61	5.94	125.90	7.18	126.87	5.66	0.48
Jaraback's sum	396.52	6.78	392.90	10.65	392.87	7.06	0.18
U1 ∧ S-N	104.61	9.07	102.40	7.83	104.60	8.41	0.09
U1 ∧ SNP-SNA	111.01	10.42	109.25	8.43	111.80	5.59	0.36
L1 ∧ Go-Me	89.57	6.06	92.25	7.53	94.40	7.84	0.08
U1 ∧ L1	129.39	9.35	133.15	12.67	125.13	11.55	0.14

Bold: significant difference between groups.

Table 3. Cephalometric measurements of the three groups reported at T0 and T1 and paired *t*-test analyses of the changes that occurred between the two time points.

	RME					SME					Control				
	T0		T1		Paired *t*-Test	T0		T1		Paired *t*-Test	T0		T1		Paired *t*-Test
	Mean	SD	*p*-Value	SD	*p*-Value	Mean	SD	Mean	SD	*p*-Value	Mean	SD	Mean	SD	*p*-Value
SNA	83.52	4.99	83.26	5.11	0.80	79.75	4.38	80.55	4.03	0.27	81.73	6.01	82.47	4.06	0.63
SNB	78.48	6.46	78.83	6.15	0.81	76.55	3.87	76.85	4.15	0.67	78.73	4.75	79.40	3.77	0.56
ANB	6.04	2.94	5.22	2.37	0.12	3.75	2.17	3.80	2.33	0.90	3.27	2.18	3.07	1.16	0.74
S-N ∧ Go-Gn	34.96	6.06	34.61	5.97	0.53	34.85	6.23	34.70	6.45	0.77	34.20	5.267	33.00	4.62	0.26
SNP-SNA ∧ Go-Gn	27.74	4.74	27.83	5.98	0.89	26.95	7.03	27.35	4.83	0.65	25.80	5.83	25.80	6.05	1.00
S-N ∧ SNP-SNA	7.61	2.84	7.04	2.99	0.36	8.25	5.29	8.25	4.36	1.00	7.07	5.75	6.60	4.05	0.78
NŜAr	124.00	5.66	124.13	5.36	0.90	123.20	7.45	124.00	7.29	0.37	121.07	7.90	122.87	6.51	0.08
SÂrGo	139.91	6.37	141.09	6.14	0.31	143.80	10.94	144.50	9.82	0.52	144.93	9.40	144.33	8.30	0.77
ArĜoGn	132.61	5.94	131.43	4.80	0.17	125.90	7.18	126.40	6.90	0.57	126.87	5.66	126.07	6.75	0.67
Jaraback's sum	396.52	6.78	396.61	5.84	0.90	392.90	10.65	394.90	9.57	0.06	392.87	7.06	393.27	5.06	0.75
U1 ∧ S-N	104.61	9.07	101.74	8.34	**0.03**	102.40	7.83	99.15	8.57	**<0.01**	104.60	8.41	104.13	7.93	0.80
U1 ∧ SNP-SNA	111.01	10.42	108.39	8.73	**0.04**	109.25	8.43	106.70	5.90	**0.033**	111.80	5.59	110.93	5.99	0.52
L1 ∧ Go-Me	89.57	6.06	93.26	7.81	**0.03**	92.25	7.532	95.10	8.21	**<0.01**	94.40	7.84	95.53	7.94	0.41
U1 ∧ L1	129.39	9.35	127.78	8.64	0.39	133.15	12.671	131.75	9.11	0.25	125.13	11.55	127.00	11.45	0.27

Bold: significant difference between groups.

Table 4. Descriptive statistics of the difference between the variables at the two time points in each group and the ANOVA test comparing the values of the three groups and the related pairwise comparisons (Bonferroni's correction).

	RME		SME		Control		ANOVA			
	Mean	SD	Mean	SD	Mean	SD	*p*-Value	RME vs. SME	RME vs. Control	SME vs. Control
Δ SNA	−0.34	4.41	0.84	3.21	0.41	4.01	0.35	0.20	0.37	0.67
Δ SNB	0.32	2.73	0.33	3.22	0.33	3.14	0.96	0.92	0.94	0.97
Δ ANB	0.63	3.24	−0.52	1.84	0.02	1.63	0.21	0.19	0.11	0.76
Δ S-N ∧ Go-Gn	−0.33	2.72	−0.24	2.33	−0.64	2.81	0.32	0.79	0.74	0.54

Table 4. *Cont.*

	RME		SME		Control		ANOVA			
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	***p*-Value**	**RME vs. SME**	**RME vs. Control**	**SME vs. Control**
Δ SNP-SNA ∧ Go-Gn	0.14	3.20	0.42	4.01	0.01	3.14	0.61	0.78	0.92	0.70
Δ S-N ∧ SNP-SNA	−0.64	3.02	0.04	3.43	0.43	2.42	0.47	0.56	0.22	0.67
Δ NŜAr	0.11	5.03	0.82	4.02	0.91	2.84	0.55	0.62	0.51	0.92
Δ SÂrGo	1.22	5.54	0.74	4.83	−0.34	5.41	0.63	0.76	0.33	0.49
Δ ArĜoGn	−1.22	4.01	0.53	3.94	−0.42	5.13	0.29	0.17	0.53	0.48
Jaraback's sum	0.13	3.42	2.01	4.11	0.24	3.41	0.71	0.10	0.90	0.11
Δ U1 ∧ S-N	3.11	9.82	−3.33	5.04	−0.21	4.93	0.46	0.02	0.29	0.04
Δ U1 ∧ SNP-SNA	2.64	10.34	−2.61	4.93	−0.43	3.61	0.13	0.11	0.42	0.11
Δ L1 ∧ Go-Me	3.69	5.72	2.94	4.31	0.62	3.73	0.61	0.16	0.92	0.06
Δ U1 ∧ L1	−1.60	8.91	−1.41	5.32	0.91	4.61	0.53	0.92	0.22	0.11

Bold: significant difference between groups.

4. Discussion

Only few studies have evaluated the cephalometric changes on the lateral cephalogram after RME and SME, and to date, only one study [21] has compared the cephalometric parameters following RME and a Ni-Ti memory screw palatal expander.

As reported by previous long-term longitudinal investigations, there are contradictory data in the literature on the effects of RME on maxillary anteroposterior positioning. Haas [22] was one of the first authors to report the presence of anterior displacement of the maxilla after maxillary expansion treatment. On the contrary, other authors did not report such displacement, instead showing the not clinically significant sagittal behavior of the maxillary bone [23,24]. The present research is in accordance with the aforementioned research, as no significant difference was noted in the three groups on the anteroposterior position of the maxilla. Concerning the SNB angle, no significant difference was found in both treated and untreated subjects, concluding that neither the RME nor the Leaf expander affected the mandibular anteroposterior positioning. These findings are in accordance with those previously published by Garib et al. [25] and other previous longitudinal studies [26–28].

Regarding the palatal plane inclination (S-N ∧ SNP-SNA), it remained unchanged in both treated and untreated groups during the entire study period. The literature shows several responses of the palatal plane secondary to maxillary expansion, from anterior–superior to anterior–inferior rotation [29–31]. Considering the lack of difference between the treated and untreated groups, it does not seem reasonable to attribute any influence in the inclination of the palatal plane to the RME.

Halicioğlu and Yavuz [21] evaluated the effects of a conventional Hyrax-type RME and a Ni-Ti memory screw with similar properties to the leaf expander to compare their sagittal and vertical dentofacial effects.

All treated subjects obtained the midpalatal sutural opening and subsequent important skeletal and dental expansions. The maxilla moved anteriorly and inferiorly in both groups, while the mandible rotated inferiorly and posteriorly much more for the Ni-Ti-screw group. The Ni-Ti screw produced maxillary expansion using lighter forces over a shorter time. The RME using the memory screw developed similar sagittal and vertical changes to those produced by the Hyrax screw.

The present study showed after RME and Leaf expander treatment a statistically significant spontaneous retraction of the upper incisors with a decreased in the U1 ∧ S-N and U1 ∧ SNP-SNA angles. These findings are in accordance with Habeeb et al. and Cerruto et al., who demonstrated a significant posterior movement of the upper incisors following RME therapy. This effect is probably due to the deciduous anchorage that permits an anterior positioning of the screw compared with appliances cemented on the first permanent molar, as already reported in previous research [32,33]. Nevertheless, no significant difference was found between the three groups.

The expansion in the anterior area can acquire more space for the upper incisors, which are then free to align spontaneously, even under the influence of the upper lip. Küçükkeleş and Ceylanoğlu [34] reported that the pressure of the upper lip on the buccal side of the upper incisors showed a significant increase after maxillary expansion but started decreasing during the retention time. Conversely, the tongue pressure generated on the lingual side of the upper incisor demonstrated a significant decrease following palatal expansion but started increasing during the retention phase. This is in accordance with Profitt [35], who reported the theory of equilibrium, which demonstrates the natural alignment and retraction of the maxillary incisors.

Similar studies reported no significant alterations for sagittal measurements [25,27]. Both treated groups and the control group presented similar behavior for maxillary and mandibular changes after the treatment and the retention period, but with no statistically or clinically significant differences. Short- and long-term vertical skeletal variations related to RME were assessed specifically for the maxilla. Garib et al. [25] reported minor changes with slight clinical significance. Furthermore, the long-term changes estimated for the mandibular plane angle were not reliable in the study of Chang et al. [25]

Unlike our study, not all previous research has considered an untreated control group. The present investigation showed no significant difference between the three groups for all the variables, but a statistically significant difference was found between T0 and T1 for the two groups of treated patients for the U1 ∧ S-N, U1 ∧ SNP-SNA and L1 ∧ Go-Me angles. This could be explained with the statistical consideration that the paired *t*-test is influenced by a small but steady difference between T0 and T1. In contrast, the one-way ANOVA test considers only the mean and standard deviation of the entire group. The result is the absence of a significant difference between the two treatment groups and the untreated controls. Mew [36] and Mutinelli et al. [37] also highlighted an improvement in dental alignment after RME. These results denote that upper incisor misalignment does not improve spontaneously with craniofacial development in the control groups, showing the same characteristics as the treated subjects before the maxillary expansion procedure was performed. Among the limitations of the study, the authors want to specify that conventional imaging is subject to bidimensional flattening, variable magnification of facial bones and overlapping of different structures. Diagnostic reliability is dependent on correct head position when taking the radiograph, and there is a need for additional teleradiographs in posteroanterior and axial projection to evaluate symmetry, thus partially hindering the conclusion that is possible to draw from this study.

5. Conclusions

The present study showed a spontaneous retraction of the upper incisors after using the RME and the leaf expander. This may be due to a pronounced expansion in the anterior area and accentuated pressure of the upper lip. Apart from the aforementioned dental variables, no statistically significant difference concerning the skeletal variables was found in any evaluated group nor between the groups. It appears that the leaf expander and the RME present similar effects such as dental and skeletal changes.

Author Contributions: Conceptualization, V.L. and C.M.; methodology, A.A.; software, A.G.; F.G.; validation, D.C., A.U. and A.A.; formal analysis, V.L.; investigation, V.L.; data curation, A.U.; writing—original draft preparation, A.A., C.M. and D.C.; writing—review and editing, C.M. and V.L.; visualization, V.L.; supervision, C.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Ospedale Maggiore Policlinico, IRCCS Cà Grandà, Milan, Italy (Protocol No.: 573/15).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data available on request due to restrictions, e.g., privacy or ethical.

Conflicts of Interest: The authors declare no conflict of interest.

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