European Journal of Musculoskeletal Diseases
ISSN 2038-4106/2021

Article

SET-UP: COMPARISON BETWEEN MANUAL AND DIGITAL METHODS

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ABSTRACT

The aim of this study is to compare the results in terms of accuracy and outcome of a group of manual set-ups with a group of set-ups performed with digital software. A clinical case of an adult patient was selected with a skeletal class I (with a slight tendency to a skeletal class III), normodivergent, with normoinclination of the upper and lower incisors. The following was performed, starting from the plaster models or digital models of the patient: 10 manual set-ups by 5 dental technicians (each dental technician repeated the set-up twice with an average interval between the first and second set-up of 2 weeks), and 10 digital set-ups by 5 orthodontists with 3Shape software (each orthodontist repeated the set up twice with an average interval between the first and second set-up of 2 weeks). Intra and inter-arch parameters were evaluated and analysed for each manual and digital set-up. The sample includes 560 pairs of measurements (TIP, TORQUE) according to the following scheme: the TIP and TORQUE of 10 manual set-ups of 28 dental elements for each tooth were evaluated; a total of 5 operators carried out the sample of 10 manual set-ups; therefore each operator performed two set-ups, the coefficient of the agreement was equal to 0.49 for the TIP and 0.37 for the TORQUE between the first test and the second test. The TIP and TORQUE of 10 digital set-ups of 28 dental elements for each tooth were evaluated; a total of 5 operators carried out the sample of 10 digital set-ups, and the concordance coefficient was equal to 0.57 for the TIP and 0.96 for the TORQUE between the first test and the second test. The average difference for TIP was greater than ($p<0.0001$) with the manual set-up (average 4.2, SD 4.6) than with the digital set-up (average 2.7, SD 2.7). The average difference for TORQUE was also higher ($p<0.0001$) with the manual set-up (average 8.1, SD 8.4) than with the digital set-up (average 3.7, SD 3.2). The digital set-up proved to be more precise than the manual set-up for all the variables examined with correct values of OB and OJ, flattened Spee and Wilson curves, coincident midlines, correct occlusal relationships, close interproximal contacts, absence of diastemas and relationships intra and inter-arch.

KEYWORDS: *orthodontic, set-up, manual, digital, diagnosis, 3Shape*

Received: 20 July 2021
Accepted: 22 August 2021

ISSN: 2038-4106

INTRODUCTION

In recent years, the orthodontic set-up has been the subject of great attention and has known many evolutions, especially in the digital era. However, it is certainly not a recent technique; it has been known and used in orthodontics for many decades (1). The first publication by Dr Harold D. Kesling in the *American Journal Of Orthodontics and Oral Surgery* dates back to 1945 and describes a technique used to build a particular device called a "Tooth Positioning Appliance". The technique of preparing a set-up, manual or digital, involves the segmentation of the dental elements and their subsequent repositioning according to the objectives of the orthodontic treatment. The execution of a set-up can have various purposes:

- represents an aid in the diagnostic phase by prefiguring the desired result;
- allows to assess the need for dental extractions;
- allows to predict the necessary interproximal reduction and its location;
- allows to assess what the distribution of spaces should be in pre-prosthetic cases and cases with agenesis;
- is used in the construction of orthodontic devices;
- allows indirect bonding technique in lingual orthodontics.

The diagnostic set-up, in some cases, represents support; in other cases, it is a fundamental and essential diagnostic instrument, it allows an accurate three-dimensional evaluation of the final objectives of the treatment, and there is no linear evaluation or measurement that can replace it (2-10).

Criteria for the execution of digital and manual set-ups

For the first time, the *Orthodontic Set Up* text by the authors G. Scuzzo, L. Lombardo and K. Takemoto clearly defines all the intra- and inter-arch dental criteria and the gnathological criteria that allow a correct set-up to be performed (11).

- The intra-arch criteria represent the objectives to be achieved within the upper and lower arches of the individual dental elements and the relationship between them: tip, torque, in and out, contact points, rotations, intercanine diameter, length and shape of the arch.
- The set-up must respect the inter-arch criteria, namely the criteria that derive from the relationship between the dental elements of one arch with those of the other: occlusal contacts, the position of the first molar, canines and incisors, overbite, overjet, the relationship between mesio-distal measurements of the elements of both arches.
- The set-up must respect the gnathological criteria: Wilson curve, Spee curve and disclusion.

Execution of the manual set-up

In the Orthodontic Set-Up text, 6 distinct phases of realisation of the manual set-up must be carried out with great accuracy and precision for the success of the result:

- execution of accurate impressions;
- creation of the plaster models of the two arches;
- separation of the dental elements;
- preparation of the articulator;
- positioning of the upper and lower arch teeth with wax;
- occlusal checks and eventual adjustments.

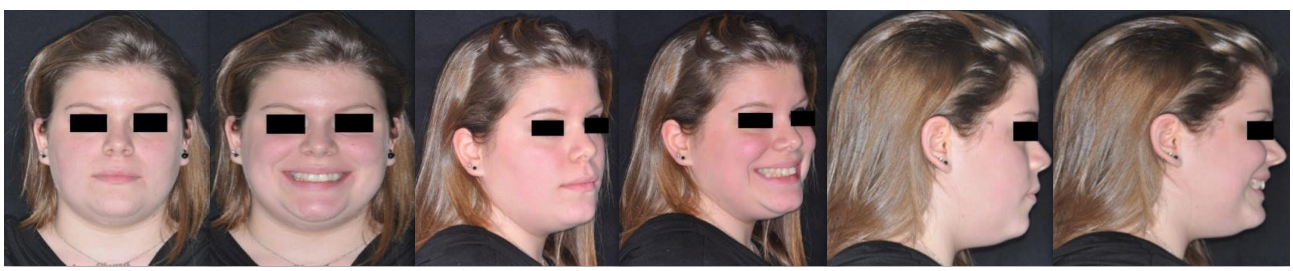

Fig. 1. *Extraoral records*

Execution of the digital set-up

The digital set-up is performed thanks to software on virtual models starting from physical plaster models subsequently scanned or from models that have been created directly by scanning the arches thanks to intra-oral scanners (12).

Here the accuracy of manual and digital set-up are compared.

MATERIALS AND METHODS

Sample selection

In the present study, we selected the case of an adult patient who presents a skeletal class I (with a slight tendency to skeletal class III), normodivergent, with normoinclination of the upper and lower incisors (Fig. 1-3).

Starting from plaster and digital models of the patient were performed: 10 manual set-ups by 5 dental technicians (each dental technician repeated the set-up twice with an average interval between the first and second set-up of 2 weeks), and 10 digital set-ups by 5 orthodontists with 3Shape software (each orthodontist repeated the set-up twice with an average interval between the first and second set-up of 2 weeks).

Execution of the diagnostic manual and digital set-up

For the execution of the manual set-ups, 5 dental technicians specialised in orthodontics were chosen and were asked to perform a total of 10 manual set-ups. The prescription request was to reach the criteria for an ideal occlusion according to the 6 Andrews keys. After performing the set-up, the dental technicians indicated whether interproximal reduction had been used and, if so, specified the location and extent. In addition, five orthodontists with experience performing digital set-ups were asked to execute 2 digital set-ups of the same patient with the 3Shape Orthoanalyzer software. For all the set-ups, STL files were created and imported into the Nemocast software and the 3shape Orthoanalyzer software for analysis.

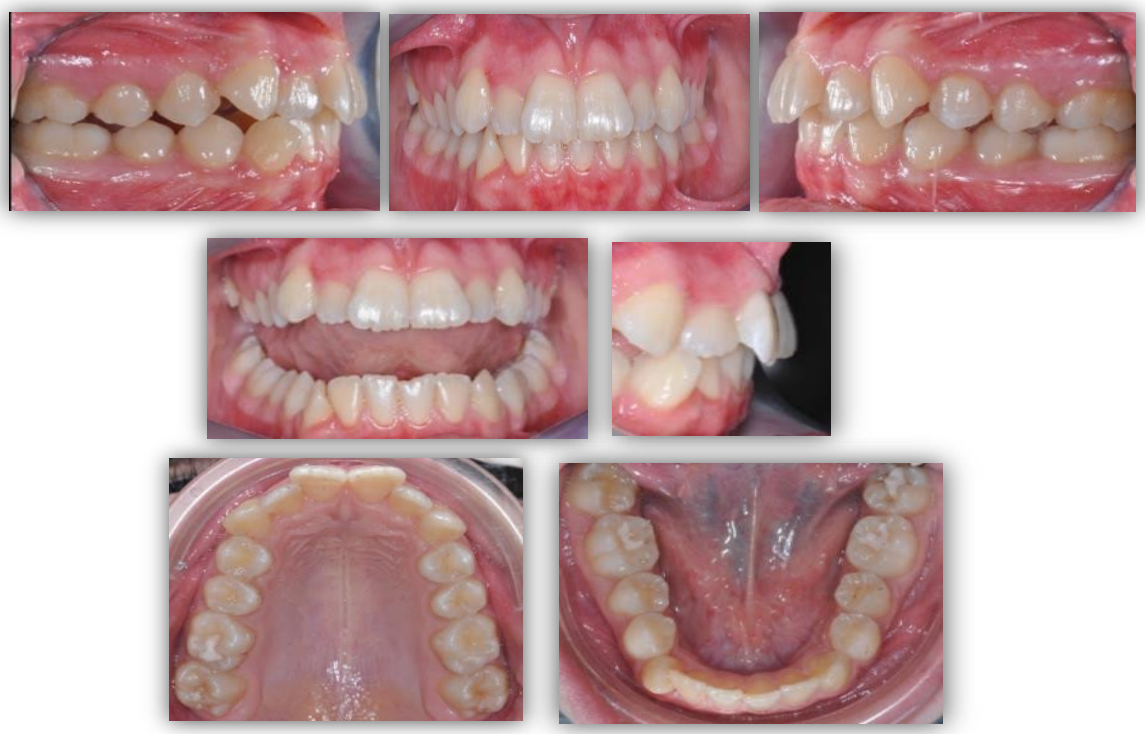

Fig. 2. *Intraoral records*

Statistical analysis

In the first approach, the numerical value of the difference was studied. A multifactorial variance model investigated the effects of technique (manual, digital) and tooth position on differences for TIP and TORQUE. In the second approach, data on the difference was reprocessed in terms of "relevant differences" (> 3 °) for each tooth (measurements that deviate from the range of Andrews' normal values). The effects of the technique (manual, digital) and the tooth position on the presence of differences detected for TIP and TORQUE were studied with a logistic model, with an estimation of the OddsRatio (OR) and the respective 95% confidence intervals (95% CI). The third approach analysed the information on "relevant differences" for each mouth. The technique's effects (manual, digital) on the number of teeth with a significant difference was studied on 28 teeth per mouth with a Poisson model. A p-value lower than 0.05 was considered significant. Statistical analysis was performed with R 3.2.2 language.

RESULTS

The sample includes 560 pairs of measurements (TIP, TORQUE) according to the following scheme: the TIP and TORQUE of 10 manual set-ups of 28 dental elements for each tooth were evaluated; a total of 5 operators carried out the sample of 10 manual set-ups; therefore each operator performed two set-ups, the coefficient of the agreement was equal to 0.49 for the TIP and 0.37 for the TORQUE between the first test and the second test. The TIP and TORQUE of 10 digital set-ups of 28 dental elements for each tooth were evaluated; a total of 5 operators carried out the sample of 10 digital set-ups, and the concordance coefficient was equal to 0.57 for the TIP and 0.96 for the TORQUE between the first test and the second test. The average difference for TIP was greater than ($p<0.0001$) with the manual set-up (average 4.2, SD 4.6) than with the digital set-up (average 2.7, SD 2.7). The average difference for TORQUE was also higher ($p<0.0001$) with the manual set-up (average 8.1, SD 8.4) than with the digital set-up (average 3.7, SD 3.2). In Table I, the results of the manual and digital techniques are reported.

Intra-arch diameters

As for the manual set-up group, in the upper arch occurs a general contraction of the arches in the middle and posterior sectors. The upper 3-3 diameter remained virtually unchanged. The lower diameters were respected except at levels 5-5, where there was a slight expansion on average. The digital set-up group is characterised by a generally more significant expansion of transverse diameters, especially at the level of the lower arch.

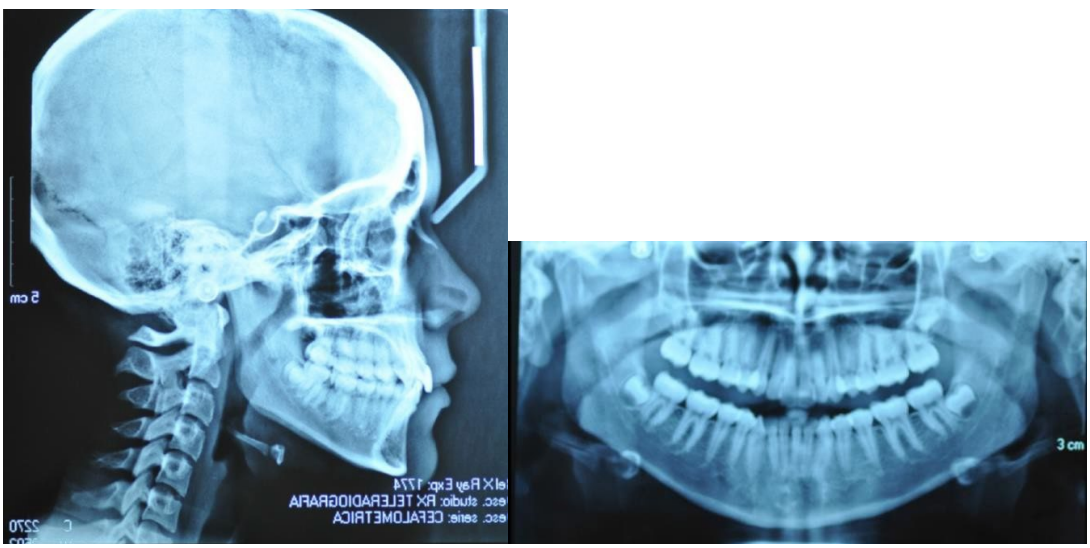

Fig. 3. *Radiological records*

Table I. *Results of the manual and digital technique*

I-Symmetry of the upper and lower arches of the setups					
MANUAL	Symmetry	DIGITAL	Symmetry		
1 a	L	**1 a**	YES		
1 b	YES	**1 b**	YES		
2 a	L	**2 a**	U; L		
2 b	L	**2 b**	YES		
3 a	YES	**3 a**	YES		
3 b	U; L	**3 b**	YES		
4 a	L	**4 a**	L		
4 b	U	**4 b**	YES		
5 a	U; L	**5 a**	L		
5 b	U; L	**5 b**	YES		
II-Spee Curve					
MANUAL	L (mm)	R (mm)	DIGITAL	L (mm)	R (mm)
1 a	0	0	**1 a**	0	0
1 b	0	0	**1 b**	0	0
2 a	2.65	1.92	**2 a**	0.7	0.6
2 b	2.1	2	**2 b**	0.6	0.5
3 a	0	0	**3 a**	0	0
3 b	0	0	**3 b**	0	0
4 a	2.4	1.88	**4 a**	0	0
4 b	2.15	2.7	**4 b**	0	0
5 a	0	0	**5 a**	0	0
5 b	0.7	0.83	**5 b**	0.5	0.5
III-Wilson Curve					
MANUAL	L (mm)	R (mm)	DIGITAL	L (mm)	R (mm)
1 a	1.61	0.73	**1 a**	1.91	1.84
1 b	1.3	1.43	**1 b**	2.3	1.45
2 a	3.66	2.74	**2 a**	1.9	1.83
2 b	3.9	3.85	**2 b**	1.78	1.89
3 a	2	1.1	**3 a**	1.81	1.63
3 b	0.9	1.2	**3 b**	1.96	1.75
4 a	2.4	1.88	**4 a**	1.58	1.32
4 b	2.36	2.8	**4 b**	2	1.49
5 a	1.92	1.81	**5 a**	1.8	1.5
5 b	1.98	2.98	**5 b**	1.66	1.85
IV-Interproximal contacts					
MANUAL	Diastema		DIGITAL	Diastema	
1 a	YES		**1 a**	NO	
1 b	NO		**1 b**	NO	
2 a	NO		**2 a**	NO	
2 b	NO		**2 b**	NO	
3 a	YES		**3 a**	NO	
3 b	YES		**3 b**	NO	
4 a	YES		**4 a**	NO	
4 b	YES		**4 b**	NO	
5 a	YES		**5 a**	NO	
5 b	YES		**5 b**	NO	

V-Arch lenght					
MANUAL	U	L	DIGITAL	U	L
1 a	44	38.5	**1 a**	42.09	37.32
1 b	44	39.5	**1 b**	42.15	37.5
2 a	44.2	38	**2 a**	42.42	37.15
2 b	44.11	38.5	**2 b**	42.12	37.36
3 a	44.51	39.21	**3 a**	42.16	37.58
3 b	44.1	38.5	**3 b**	41.87	37.61
4 a	44	39.19	**4 a**	42.59	37.3
4 b	44.81	38.98	**4 b**	42.64	37.63
5 a	44	38.08	**5 a**	41.9	37.37
5 b	42.8	37.1	**5 b**	42.21	36.9
Average	44.053	38.556	Average	42.215	37.372
St.Dv.	0.51	0.71	St.Dv.	0.26	0.22
VI-Interproximal reduction					
MANUAL	IPR	DIGITAL	IPR		
1 a	NO	**1 a**	YES		
1 b	NO	**1 b**	YES		
2 a	NO	**2 a**	NO		
2 b	NO	**2 b**	NO		
3 a	YES	**3 a**	YES		
3 b	YES	**3 b**	YES		
4 a	YES	**4 a**	YES		
4 b	NO	**4 b**	YES		
5 a	YES	**5 a**	NO		
5 b	YES	**5 b**	NO		
VII-Overjet and Overbite					
MANUAL	0B (mm)	OJ (mm)	DIGITAL	0B (mm)	OJ (mm)
1 a	2.7	3.5	**1 a**	1.7	1.9
1 b	2.1	3.2	**1 b**	2.4	2.1
2 a	3.7	4.5	**2 a**	2.3	2.3
2 b	4.2	2.9	**2 b**	2.7	2.2
3 a	2	2,9	**3 a**	2	1.5
3 b	3.2	4	**3 b**	1.7	2.1
4 a	3.9	3.9	**4 a**	1.9	2.5
4 b	3.7	3.3	**4 b**	2	2
5 a	2.1	2.2	**5 a**	1.3	2.3
5 b	2.3	2.4	**5 b**	2.3	2.2
Average	2.99	3.28	Average	2.03	2.11
St.Dv.	0.84	0.72	St.Dv.	0.84	0.72

Canine and molar class

In the manual set-up group, 7 out of 10 cases remain an incomplete class correction at the molar or canine level, with a slight tendency to a skeletal class II. On the other hand, the digital set-up group showed the class correction at the canine and molar levels in all cases.

Midlines

The midlines are all correctly centred in the digital set-up group, while in the manual set-up group, 4 out of 10 showed various degrees of deviation.

DISCUSSION

From the bibliographic review, several studies have been published in which digital set-ups were used to aid the formulation phase of the treatment plan; only two studies compare the digital set-ups with the manual set-ups and evaluate their precision. The first study, published in AJODO by Korean researchers (12), compares the digital set-ups with the manual set-ups in 10 extraction cases; for each patient, a manual and a digital set-up was performed. This study concludes that there is no significant difference between manual and digital set-ups between intra-arch measurements and inter-arch occlusal variables. This data contrasts with the results of the present study in which the digital set-up technique proved to be more precise for all the variables examined. These differences can be explained at least in part by operator-dependent reasons. In the study carried out by Korean researchers, the manual and digital set-ups were carried out by the same operator, while the present study considered the manual and digital set-ups made for a single case by different operators.

The second study comparing the digital set-up with the manual set-up was published in 2015 by a group of Brazilian researchers (13); this work examines the cases of 20 adult patients who had already completed the treatment for each patient with a digital set-up and a manual set-up were performed which were compared with the final models of the patients upon completion of the orthodontic treatment. Only three linear measurements were made: intercanine diameters, intermolar diameters and arch length. The study revealed no significant differences between the measurements, indicating that digital set-ups are equally effective and accurate as diagnostic and treatment planning tools. In this study, only a few linear measurements are considered without evaluating all the intra- and inter-arch parameters that define the result from a qualitative point of view of a set-up.

According to a study (14), the measurements performed on digital 3D models represent valid and reliable alternatives to those performed on physical models with a significant advantage in reduced execution times. Furthermore, according to Sousa et al., digital models were reliable and comparable to physical models to obtain the most common measurements in orthodontic diagnostics (15).

CONCLUSIONS

The digital set-up group show the TIP and TORQUE values, on average, more correct and close to the standard value in a statistically significant way.

The digital set-up proved to be more precise than the manual set-up for all the variables examined with correct values of OB and OJ, flattened Spee and Wilson curves, coincident midlines, correct occlusal relationships, close interproximal contacts, absence of diastemas and relationships intra and inter-arch.

The manual set-up group saw a strong decrease in the mesio-distal diameters of the elements and the presence of diffuse diastematures, suggesting that the separation of the dental plaster elements did not occur correctly and the interproximal anatomy was not respected.

The digital set-up allows for overcoming some important limitations of the manual method, minimising the possibility of introducing errors during the process and allowing the orthodontist to play a leading role in its execution.

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European Journal of Musculoskeletal Diseases
ISSN 2038-4106/2021

Article

SOFT AND HARD TISSUE CHANGES FOLLOWING MANDIBULAR SETBACK SURGERY IN SKELETAL CLASS III PATIENTS

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ABSTRACT

Skeletal class III patients are often treated with surgical and orthodontic treatment to balance the facial profile and improve facial aesthetics. According to previous studies, the primary motivation of patients for orthodontic treatment along with jaw surgery has been to improve their aesthetic condition. Therefore, improving the patient's profile is one of the important goals of surgical treatments. Here, 16 skeletal class III patients were orthodontically and surgically treated. In addition, lateral radiograms performed at the beginning and end of treatment were compared to get information regarding soft tissue modification. According to the results of this study and its comparison with the literature, changes in the soft tissue are related to the amount of mandibular setback: the higher the setback, the greater changes occur in the profile. This fact has a significant impact on aesthetics and patients' expectation.

KEYWORDS: *mandible, osteotomy, profile, aesthetic, expectation*

Received: 16 July 2021
Accepted: 23 August 2021

ISSN: 2038-4106

INTRODUCTION

Skeletal class III patients are often treated with a combination of surgical and orthodontic treatment to balance the facial profile and improve facial aesthetics (1). Given the advances in orthognathic treatment techniques over the past decades, these combination therapies have been widely used to correct moderate to severe skeletal problems (2). According to previous studies, the main motivation of patients for orthodontic treatment along with jaw surgery has been to improve their aesthetic condition (3, 4). Therefore, improving the patient's profile is one of the important goals of surgical treatments, and the treatment plan for skeletal class III patients should not only consider the function but should also include considerations related to facial aesthetics (5, 6).

Unlike orthodontic treatments, which cause gradual changes in the patient's appearance, combined orthodontic and surgical treatments cause sudden and drastic changes that require rapid and immediate psychological adjustment of the patient's self-perception to these changes (7-9). Therefore, in treating these patients, the clinician should be able to analyze and predict soft tissue changes after surgery with different methods (10-12). Conventional use of normal values of two-dimensional cephalometry can guide practitioners in diagnosing and treating these cases and predicting the results of hard and soft-tissue after surgery (13). Numerous studies have evaluated hard and soft tissue changes after mandibular setback surgery in skeletal class III patients that are conflicting in their findings (14-17).

The aim of this study is to evaluate soft tissue changes after mandibular setback based on cephalometric radiography performed before and after surgical treatment of class III patients.

MATERIAL AND METHODS

The study was conducted according to the guidelines of the Helsinki Declaration of 1975, revised in 2013. The approval code of the present cross-sectional study was obtained from Tekovo University (n. 1381). All the participants signed approved written consent.

Sixteen patients (13 females and 3 males) referred to the orthodontic department were selected according to the following criteria: skeletal class III based on lateral cephalometry and clinical examination; no history of previous orthodontic treatment and presence of any syndrome.

Presurgical lateral cephalometry, orthopantomography, photographs and dental cast were collected. The following measures were recorded:

LS-SIE (*labial superior-superior incisal edge*) and LI-IIE (*labial inferior-inferior incisal edge*) in soft tissue;

the lower third of the facial height (S.N-PT), mandible length (Go-Gn) in hard tissue;

SNA, SNB, and NLA angles; and

Soft tissue thickness: A-Sn, U.L.L. (*Upper Lip Length*), LLL (*Lower Lip Length*), and chin thickness were measured.

Pre-orthognathic surgery procedures (levelling, aligning, and functional compensation) were performed with 0.022 slot edgewise appliances up to 0.019 * 0.025 stainless steel archwire to prepare the patients. Then, bilateral sagittal Osteotomy was carried out by the surgeon. Finally, postsurgical orthopantomography and lateral cephalometry (after 6 months) were done.

Statistical analysis

The collected data were analyzed by T-test, Paired T-test, using Statistical Package for the Social Sciences software version 19 (Chicago, IL, USA). A P-value of less than 0.05 was considered significant.

RESULTS

In 16 skeletal class III patients there had the following changes after mandibular setback:

- regarding soft tissues modification, LI-IIE (labial inferior-inferior incisal edge) index increased by 0/2 degree (14/6%) (P<0/005), whereas LS-SIE (labial superior-sup incisal edge), did not change significantly (Table I);
- regarding hard tissue modification, the lower third of the facial height increased by 0/1 mm (P<0/2); the mandible

length (Go-Gn) was reduced by 0/7 mm (P<0/001) (Table II);

- as regards angles' change, the mento-labial (ML) angle had a significant reduction of 8/7 degrees (P<0/01), as well as the angle of SNB decreased by 3/3 degrees (P<0/001), whereas the SNA angle raised 0/8 degrees without reacting a statistically significant p-value (Table III);
- as regards soft tissue thickness, the chin soft tissue shrinkages for 0/03 mm postoperatively (without significant p-value), while ULL (Upper Lip Length) increased by 0/1 mm (P<0/007) and the LLL (Lower Lip Length) decreased by 0/2 mm (P<0/01) (Table IV).

Table I. *Soft tissue changes pre- and post-operatively (in mm) in Class lll malocclusion*

	LS-SIE Mean ± SD	LI-IIE Mean ± SD
Pre-operatively (mm)	1/8 ± 0/3	1/6 ± 0/3
Post-operatively (mm)	1/7 ± 0/3	1/8 ± 0/3
The difference (%)	0/5%	14/6%
P-value	0/01	0/005

Table II. *Hard tissue changes pre and post operatively (in mm) in Class lll malocclusion*

	Sn.PT*(lower third facial height)* Mean ± SD	Go.Gn Mean ± SD
Pre-operatively(mm)	6/9 ± 0/8	8/2 ± 0/8
Post-operatively(mm)	7/1 ± 0/8	7/5 ± 0/8
The difference (%)	1/6%	8/4%
P-value	0/2	0/001

Table III. *The angle's changes pre- and post-operatively (in degrees) in Class lll malocclusion*

	NLA *(nasolabial angle)* Mean ± SD	SNA Mean ± SD	SNA Mean ± SD
Pre-operatively (degrees)	117 ± 10/5	83/7 ± 3/9	78/5 ± 20
Post-operatively (degrees)	113/1 ± 13/9	80/4 ± 4/4	79/3 ± 20
The difference (%)	3/3%	4%	1%
P-value	0/2	0/001	0/6

Table IV. *The changes of the soft tissue thickness pre and post operatively (in mm) in Class lll malocclusion*

	A.Sn Mean ± SD	L.L.L Mean ± SD	U.L.L Mean ± SD	U.L Thickness Mean ± SD	A-Sn/PT-Pog[1] Mean ± SD	Li-IIE/PT-Pog Mean ± SD	Ls-SIE/PT-Pog Mean ± SD
Pre-operatively (mm)	1/67 ± 0/4	2/3 ± 0/3	2/2 ± 0/3	16/7 ± 2/9	1/8 ± 0/6	1/6 ± 0/3	1/89 ± 0/5
Post-operatively (mm)	1/73 ± 0/4	2/1 ± 0/3	2/1 ± 0/25	17/1 ± 3/4	1/9 ± 0/5	1/9 ± 0/5	1/86 ± 0/5
Difference (%)	2/4%	8/6%	4/5%	2/4%	5/5%	18/8%	2/1%
P-value	0/7	0/01	0/007	0/7	0/2	0/002	0/7

1PT-Pog: soft tissue thickness of chin

DISCUSSION

Although significant advances have been made in predicting hard tissue changes following orthognathic surgery, this is not true regarding soft tissue predictability. The response of soft tissue to hard tissue movements following surgery varies among patients.

Changes in soft tissue compared to hard tissue were studied for the first time by McNeill et al. (13). Soft tissue response after orthognathic surgery may be influenced by preoperative variables such as deformity, soft tissue thickness, and muscle tonicity (14, 15). The thicker the soft tissue, the less it is affected by hard tissue movements (15, 16). Other factors include degree of dissection, hematoma, edema, incision suture, scar formation, and tissue contraction (17, 18). Some of these factors are controllable, which can lead to more predictable outcomes after surgery. However, due to postoperative oedema, soft tissue results should be evaluated at least 6 months after surgery (19, 20).

This study evaluated soft and hard tissue changes following the mandibular setback in skeletal class III patients. The mento-labial angle was reduced by 7.8 degrees, meaning that the mento-labial sulcus's concavity was increased, and the sulcus became deeper. This finding, according to presurgical decompensation of lower incisors, seems rational, and it is consistent with the study of Kim et al. (10).

Hu et al., (14), declared that the soft tissue thickness of the chin related to hard tissue was increased by 0.7 in men and 0.6 mm decreased in women following mandibular setback. According to our findings, the LI-IIE index increased 0/2 degree (14/6%), while Jensen, et al. reported a 5/3 mm change (21). In addition, the ULL increased by 0/1 mm significantly, and the LLL decreased by 0/2 mm significantly based on our study, and these results were similar to the results obtained by others (21, 22).

We found that upper and lower soft tissue thickness did not change significantly; Gjørup et al. (23), explained that changes in upper and lower lip soft tissue thickness are related to initial preoperative thickness, and it is closely correlated with the amount and direction of hard tissue movement after mandibular surgery.

In the present study, the lower third facial height (Sn-Pt) did not change significantly following mandibular setback surgery, while Gaggl et al (24). reported a 0.2 increase in lower third facial height after mandibular setback. However, the soft tissue response does not follow rigid rules, and only general conclusions can be drawn; more research will be needed due to the variety of individual variations and influencing factors (25, 26). Further research may focus on the understanding of chewing movements in Class II patients after surgery (27) using artificial intelligence whose potentiality will be increased in the next future (28, 29).

CONCLUSION

According to the results of this study and its comparison with the literature, changes in the soft tissue are related to the amount of mandibular setback: the higher the setback, the greater is change occurring in the profile, which has a significant impact on aesthetic and patients' expectations.

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European Journal of Musculoskeletal Diseases
ISSN 2038-4106/2021

Case-study

RESOLUTION OF A CASE OF POST-TRAUMATIC ELBOW BURSITIS WITH OXYGEN-OZONE THERAPY

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ABSTRACT

Elbow bursitis is an inflammatory disease of the synovial bursa which is located near the olecranon of the ulna. The authors report a case of post-traumatic elbow bursitis treated and completely resolved in a very short time and without any side effects with oxygen-ozone infiltrative therapy.

KEYWORDS: *oxygen, ozone, elbow bursitis, medical ozone*

INTRODUCTION

Under normal conditions, the bursa of the elbow appears to have a flattened shape, but in cases of inflammation, it swells due to the increase of fluid inside it. Nowadays, in addition to specific direct causes, various risk factors have been recognized, which can increase the chances of their occurrence.

The direct causes are trauma to the elbow, prolonged pressure in the elbow, infections following cuts, wounds, or insect bites at the olecranon level.

The risk factors for olecranon bursitis are:

Elbow surgery: including minimally invasive operations such as arthroscopies;

Major traumas such as fractures: the most frequent bone injuries for this joint are the fracture of the olecranon, the fracture of the radial head, and the distal fracture of the humerus;

Forms of rheumatic diseases: as in the case of rheumatoid arthritis or gout;

No apparent cause: a small percentage of patients develop elbow bursitis without any currently known reason.

This condition is characterized by the appearance of various symptoms, not to be confused with those deriving from

Received: 10 June 2021
Accepted: 12 August 2021

ISSN: 2038-4106

epicondylitis, which may be present concomitantly. Symptoms and signs are:

Local swelling: the swelling in severe cases can have a size of several centimeters, similar to that of a ping pong ball;

Heat: in cases where there is an acute inflammatory state (with or without infection);

Pain: present both on palpation of the bursa and on movement, especially in the degrees of maximum flexion, since the bursa is stressed in that position. Pain is usually local, but in some cases, it radiates downwards, along the forearm, and upwards along the arm. Pain is often associated with a burning sensation.

Reduction of strength, especially in the extension movement;

Reduction of joint function: difficulty performing many normal daily activities, particularly resting the elbow on a rigid surface (1-14).

Treatment

The classic treatment of post-traumatic elbow bursitis differs according to the severity of the clinical picture. If the bursitis is mild, it is usually sufficient to use an ice pack, observe a rest period, combine an anti-inflammatory drug to reduce inflammation and pain, and a compressive elastic bandage to contain the discomfort caused by the movements. In addition, it is useful to associate physiotherapy that has no contraindications or side effects.

In most severe cases, it is necessary to aspirate the synovial fluid contained in the inflamed bursa and proceed with the infiltration of corticosteroids directly into the bursa to extinguish the inflammation and reduce the risk of it forming again. If there is a septic state with purulent material in the bursa, both intrabursal and systemic antibiotic drugs should be used. In cases where these therapeutic approaches fail, surgical removal of the bag must be performed (1 ,6, 7, 8, 10).

CASE REPORT

A mixture of Oxygen Ozone (O_2-O_3) is used locally through infiltrations and systemically using the venous and/or rectal routes (through insufflations).

The treatment must be adapted to the pathology to be treated by changing the concentration of ozone. For this, special devices must be used (suitably certified for medical use) which produce the mixture of Oxygen Ozone (O_2-O_3), starting from pure oxygen. The treatment is safe, has no local effects, and has few contraindications.

In order to produce the oxygen-ozone mixture, a Maxi Ozon Active Generator Device (Medica S.r.L., Bologna, Italy) was used, equipped with a digital photometer for the regulation of ozone concentrations and with check valves for the collection of the gaseous mixture in absolute sterility (15-24).

Clinical case

M.B. is a 59-year-old male, developed bursitis following repeated trauma to his left elbow with local pain and hot and red skin. The ultrasound examination carried out immediately after the appearance of the swelling of the olecranon bursa confirmed the diagnosis of left elbow bursitis (Fig. 1).

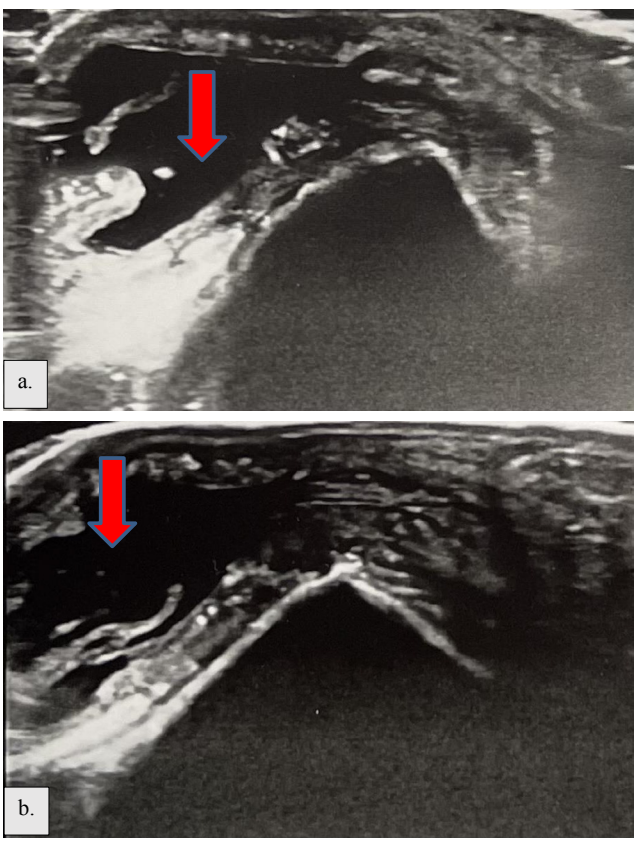

Fig. 1. ***A-B)*** *Ultrasound: effusion into the olecranon bursa (arrows).*

A minimally invasive treatment with oxygen-ozone (O_2-O_3) was proposed to the patient, who signed the consent form. Three therapeutic sessions delayed by 7 days from each other were scheduled. The treatment was performed

using 18 G pink code needles. Infiltration of the olecranon bursa does not cause particular pain. Therefore we can use needles of a slightly higher caliber than other oxygen-ozone treatments without the need for a local anesthetic, after local disinfection with betadine (Fig. 2-5).

RESULTS

The patient had a complete recovery 7 days after the end of the therapy (Fig. 6).

DISCUSSION

The classic treatment of post-traumatic elbow bursitis differs according to the severity of the clinical picture. If the bursitis is mild, it is usually sufficient to observe a period of rest, combine an anti-inflammatory drug to reduce inflammation and pain, and use an ice pack and a slightly compressive elastic bandage to limit movement.

In the most severe cases, it is necessary to aspirate the synovial fluid contained in the bursa and proceed with the infiltration of corticosteroids directly into the bursa to stop inflammation and reduce the risk of relapse.

In addition to drugs, bursitis therapy can also include applications of local physical therapies (such as laser therapy, cryotherapy, or ultrasound). In some more severe cases, especially if relapsing or difficult to resolve, surgical removal of the inflamed bursa may be indicated (15-24).

In cases with no clear traumatic origin (direct or from repeated trauma), it is essential to exclude any concomitant pathologies to be treated (e.g., gout or rheumatoid arthritis). Since the joint effusion was already significant in the case treated by us, it was necessary to empty the olecranon bursa.

Six cc of blood serum was aspirated, and 5-6cc of the mixture of O_2-O_3 at a concentration of 16 µg / ml was immediately injected (using the same needle). We decided to treat the patient with oxygen-ozone instead of the standard corticosteroid therapy to avoid side effects that often follow such infiltrations.

In the reported case, from the first session, the clinical improvement was noticeable, with the almost total disappearance of the painful symptoms and consequent better joint mobility. At the second session, after two days, the aspiration of serum-blood fluid was decidedly less than 3 cc. Furthermore, in this case, 5/6 cc of the oxygen-ozone mixture at 16 µg / ml were infiltrated, while, after a further 2 days, at the third treatment, there was no longer any need to empty the olecranon bursa. Finally, at the clinical check-up seven days after the last treatment, there was no longer any sign of bursitis

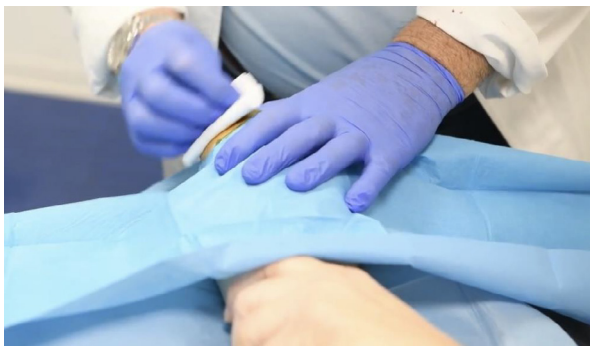

Fig. 2. *Disinfection of the olecranon bursa.*

Fig. 3. *Puncture of the olecranon bursa with an 18G needle.*

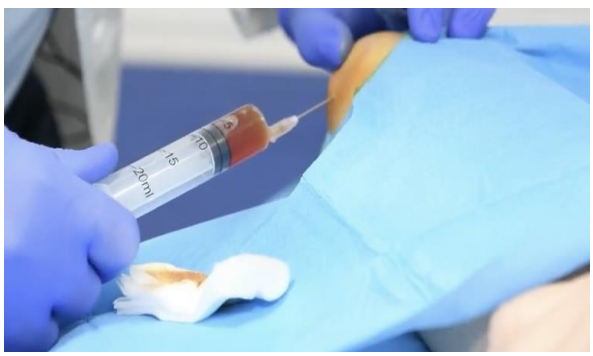

Fig. 4. *Aspiration of the blood serum liquid present in the olecranon bursa that is proof of post-traumatic genesis of bursitis.*

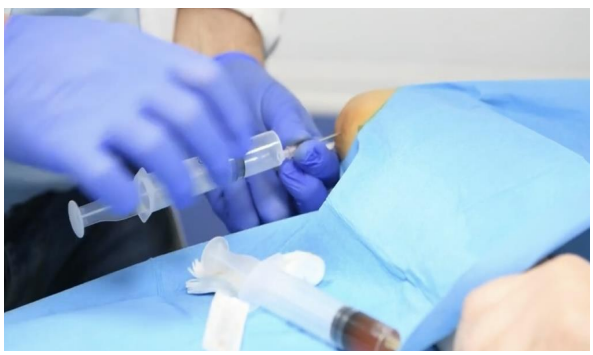

Fig. 5. *Infiltration of the bursa with an oxygen-ozone mixture at 16 µg /ml.*

treated with complete resolution of the painful symptoms, with normal skin and no longer red.

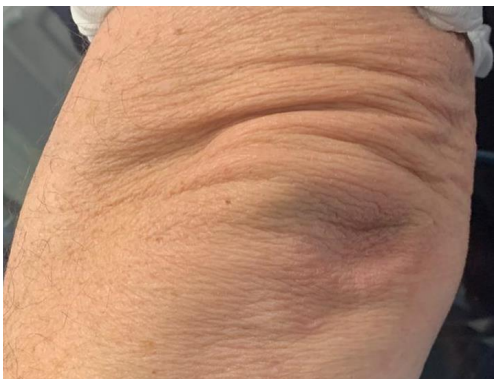

Fig. 6. *Check-up 7 days after the end of the treatment.*

CONCLUSIONS

Based on the reported clinical result, although this is a single case, we believe that oxygen-ozone therapy is an extremely effective, fast, safe therapy, free of side effects and contraindications. In conclusion, we recommend considering the treatment of the olecranon bursa with oxygen-ozone therapy as the first therapeutic approach in the case of post-traumatic elbow bursitis.

Conflict of interest

The authors declare that they have no conflict of interest.

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European Journal of Musculoskeletal Diseases
ISSN 2038-4106/2021

Review

PAIN IN FIXED ORTHODONTIC TREATMENT. ROLE OF PHOTOBIOMODULATION: DREAM OR REALITY?

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ABSTRACT

Pain is an unpleasant emotional and sensory experience. For many years orthodontists have been looking for an effective method of reducing this feeling of discomfort. As a result, Photobiomodulation (PBM) has recently taken hold in the orthodontic field. Among the countless advantages, it can modulate the painful feeling. The aim of this research is to identify the use of photobiomodulation in subjects undergoing fixed orthodontic treatment, to reduce the pain and discomfort it causes. The research was conducted from the Web of Science, Pubmed and Scopus databases. Only 14 articles met the inclusion and exclusion criteria and were used to conduct the research. The different studies compared, in most cases, patients whose mouth was divided into a part treated with laser therapy and a placebo part. The results show a statistically significant difference in perceived pain between the irradiated and non-irradiated arch. Three authors did not find statistically significant results in favour of low-laser therapy, but it is important to remember that they used different parameters. To obtain generally valid studies with consistent and reproducible results, it is necessary to standardise the different parameters independent of the operator performing the procedure.

KEYWORDS: *photobiomodulation, low-level laser therapy, pain, orthodontic treatment*

INTRODUCTION

Pain is an unpleasant emotional and sensory experience. It is known that it is one of the negative aspects of a fixed orthodontic treatment. It is perceived as discomfort, dull pain, and hypersensitivity in affected teeth (1, 2) and is present in most procedures: separator placement, banding, initial wire engagement, wearing elastics and debonding (3, 4, 5).

About 90% of patients undergoing orthodontic treatment experience a painful sensation (6), and according to O'Connor, it is considered the fourth most frequent reason for apprehension and fear in patients who need to start any fixed treatment (7).

The pain associated with orthodontic appliances is a real problem for the patient since it interferes with chewing

Received: 02 July 2021
Accepted: 25 August 2021

ISSN: 2038-4106

performance and speech (8).

For many years, orthodontists have searched for an effective method to reduce the discomfort their patients perceive, as this often determines whether to continue therapy (9).

In recent years, Photobiomodulation (PBM) has taken hold in the orthodontic field for the countless advantages it brings: it can induce the activation and proliferation of osteoblasts and osteoclasts, therefore accelerating the remodelling of bone, increase the velocity of orthodontic tooth movement (10, 11, 12) and the efficiency of orthodontic treatment during dental alignment (13); it can also be used to enhance keratin synthesis (14, 15), in case of hypersensitivity, analgesia and inflammatory processes in periodontal tissues (16), but above all, it can modulate the painful feeling as a non-invasive, non-thermal and inexpensive technique without significant adverse effects (17, 18).

PBM's analgesic and anti-inflammatory properties are attributed to increased blood flow, decreased prostaglandin E2 and inhibiting COX-2 enzyme secretion (19, 20).

The aim of this research is to identify in the literature the use of photobiomodulation in subjects undergoing fixed orthodontic treatment to reduce the perception of pain and discomfort that it causes.

MATERIALS AND METHODS

The research was conducted from the Web of Science, Pubmed and Scopus databases. Hand-searching was not performed. Keywords used were "photobiomodulation", "laser", "orthodontic", "dental movement", "tooth movement", and "pain". Inclusion and exclusion criteria were determined prior to reading the retrieved abstracts.

The inclusion criteria were as follows:

- Articles published in the last 10 years,
- Studies published in the English language,
- Studies conducted on the human species,
- Participants that underwent fixed orthodontic treatment without limitation in gender, age, race and socio-economic status,
- Randomised clinical trials which analysed the effectiveness of PBM in reducing orthodontic pain compared with a placebo group (simulated pain treatment) and/or a control group (no treatment of any kind),
- Studies that used the analogue visual scale (SEA), the numerical evaluation scale or another type of questionnaire to evaluate the duration and intensity of pain.

The exclusion criteria were as follows:

- Articles not written in the English language,
- Studies were cases or letter reports, review articles, cohort studies, opinion articles, abstract and descriptive,
- Studies in vitro studies or animal,
- Participants had pain caused by acute or chronic dental, periodontal or gum disorders,
- Studies of patients compromised by neurological and psychiatric disorders, systemic diseases or chronic pain,
- Participants not subjected to fixed orthodontic treatment such as studies on orthodontic elastomeric separation or similar.

The articles found in the search were selected based on their abstract, title and keywords relevance. Publications addressing relevant questions were read in full and either included for further analysis or excluded.

RESULTS

Three hundred twenty-one results have been identified through database searching: 164 on Web of Science, 71 on Pubmed, and 86 on Scopus. The filters "last 10 years" and "human species" have been applied, finding the following articles: 136 on Web of Science, 52 on Pubmed and 63 on Scopus. After excluding duplicates and reviewing titles and abstracts, 52 articles were evaluated in full text. Only 14 articles met the inclusion and exclusion criteria and were used to conduct the research.

The selected studies that evaluated the effectiveness of PBM for orthodontic pain used different parameters, such as wavelength, power output, energy dose, exposure duration, focal spot area, power density, energy density, and frequency of treatment. Moreover, the subjects examined differ in age, sex, cultural difference, malocclusion and more. Table I

shows the different parameters of each study.

DISCUSSION

For years orthodontic treatment has been accompanied by pain, considered natural and negligible compared to possible problems such as prolonged treatment time, periodontal problems and root resorption (21).

To date, more and more orthodontists are looking for a way to relieve patients' pain. There are several ways to decrease this discomfort, such as using drugs, chewing plastic wafers or gum, eating a diet of softer foods, and using vibratory and transcutaneous electrical stimulation (22, 23).

PBM is one of the latest methods to relieve orthodontic pain. Although the mechanisms of action are not yet clear (16), low-laser therapy has been shown to have neural and anti-inflammatory periodontal regenerative properties. The use of a diode laser in a continuous wave can significantly reduce pain after tooth movement in the first three days (24, 25).

Orthodontic treatment

Table I. *The different parameters of each study.*

Articles	Study design	Laser	Wavelength and power	Dose	Total energy	Pain measurement	Subject	Orthodontic treatment
Dominguez et al. (2013)	Single-blind RCT (split mouth)	GaAlAs laser	830 nm 100 mW	80 J/cm2, 2.2 J vestibular and palatal surface, for 22 sec each Only one dose: T0	4.4 per tooth	VAS after 2 h (T1), 6 h (T2), 24 h (T3), 2 days (T4), 3 days (T5), and 7 days(T6)	59: 40 F - 19 M AGE: 20-30	mini brackets Equilibrium and self- ligating brackets slot 0.022 inch
Wu et al. (2018)	Double-blinded RCT	GaAlAs diode laser	810 nm 400 mW	2 J/cm2 3 points/side, for 20 sec each Multiple doses: 0h, 2 h, 24 h, 4 d, and 7 d	Not indicated	Quantitative sensory testing (QST) at 0 h, 2 h, 24 h, 4 d, and 7 d	40: 30 F – 10 M Age: 12-33	self-ligating brackets slot 0.022 inch
Sobouti et al. (2015)	Single-blind RCT (split-mouth) placebo-controlled	He-Ne laser	632.8 nm 10 mW	6 J/cm2 buccal and palatal: radical apical for 80 sec and coronal for 40 sec Only one dose: T0	Not indicated	VAS on the 1, 2, 4, and 7 days	27: 11 F – 16 M Age: 12-21	metal pre-adjusted brackets (Extractions)
Isola et al. (2019)	RCT (split mouth)	Diode laser	810 nm 1 W	66.7 J/cm2, 3 points/side for 15 sec each Multiple doses: 0d, 3d, 7d, 14d and every 15d	8 J (2 x 40 s x 100 mW)	VAS at 3, 7, and 14 days	41: 20 F – 21 M Age: 10-18	metal brackets slot 0.022–0.028 inch (Extractions)
Qamruddin et al. (2017)	Single-blinded RCT (split-mouth)	GaAlAs diode laser	940 nm 100 mW	7.5 J/cm2, 5 points/side, 3 sec for each point Multiple doses: T0, T1 and T2	Not indicated	NRS 4h and 24h after each application	20: 10 F – 10 M Age: 12-25	self-ligating MBT brackets slot 0.022-inch (Extractions)
Celebi et al. (2019)	RCT (split-mouth)	GaAlAs diode laser	820 nm 110.3 mW	1.76 J/cm2, 3 points/side for 16 sec each. Only one dose	Not indicated	VAS 2h, 6h, 24h, 2d, 3d and 7d	60: 30 F – 30 M Age: 11-23	fixed orthodontic tratment, slot 0018x0.025 inch
Domiguez A. et al (2013)	RCT	Diode laser	670 nm 200 mW	6.37 W/cm2, 3 surface, 3 min on each surface Multiple doses: 0, 1, 2, 3, 4, and 7 days	108 J	VAS day 0, 1, 2, 3, 4, 7, 30, and 45	10: 5 F – 5 M Age: 12-16	fixed orthodontic treatment slot 0.018 inch (Extractions)
Qamruddin et al. (2018)	single-blinded RCT (split mouth), placebo controlled	GaAlAs diode laser	940- nm 100 mW	7.5 J/cm2, 5 points/side for 3 sec. Only one dose	75 J per tooth	NRS. at consecutive 12 h intervals for 7 days	42: 26 F – 16 M Age: 12-25	Fixed orthodontic treatment slot 0.022-inch (Extractions)
Doshi-Mehta et al (2012)	RCT (split mouth)	GaAlAs diode laser	800 nm 0.7 mW	8 J (2 x 40 sec x 100 mW). 5 points/side Multiple doses: 0, 3, 7, and 14 days	8 J (2 x 40 s x 100 mW).	Visual pain scale at 1, 3, 30 days	20: 12 F – 8 M Age: 12-23	fixed orthodontic treatment slot 0.022-inch (Extraction)
Storniolo-Souza et al. (2020)	double-blind, placebo controlled RCT (split mouth)	ArGaAl-Twin Laser	780 nm 40-70 mW	10-35 J/cm2 5 points/side 10-20 sec each Single monthly dose	4 J for mandible 9 J for the maxilla	VAS at12, 24, 48 and 72 hours	11 Age: ±14	Fixed appliances slot 0.022 × 0.028 inch (Extraction)
Lo Giudice et al. (2019)	RCT	diode laser	980 nm 1 W	24-27 J/cm2 A total of 50 sec Multiple doses: 3 times at intervals of 2 min	150 J/cm2 for mandibular arch	NRS at 2h, 6h, 24 h, from day 2 to 7	84: 43 F – 41 M Age: 16.5±2.8	self-ligating appliance slot 0.022 inch
Alam MK. (2019)	Prospective clinical intervention	GaAlAs laser	940 nm 100 mW	7.5 J/cm2 5 points/side for 3 sec each Only one dose	75 J per tooth	NRS At 4 h, 24 h, 3 d, and 7 d	32 F>M Age: 14-25	Conventional backets and self-ligatin brackets slot 0.022 inch
Al Sayed Hasan (2020)	single-blind, placebo-controlled, RCT	GaAlAs laser	830 nm 150 mW	4.25 J/cm2 2 point/side for 15 sec for each tooth Only one dose	2 J per point	VAS At 1, 6, 24, 48, and 72 h	26 Age 16-24	fixed orthodontic treatment (Extraction)
Guram et al. (2018)	RCT double-blind splint-mouth	Ga-Al-As laser	810 nm 0.2 W	5 J/cm2 8 spots for 10s Multiple doses: each week for 21 days	Not indicated	Wong-Baker Faces Rating Scale days 1 to 7	20 12 F – 8 M Age: 17-24	fixed orthodontic treatment MBT bracket 0.022 inch (Extraction)

RCT: Randomized Clinical Trial; VAS: Visual Analogue Scale; QST: Quantitative Sensory Testing; NRS: Numerical Rating Scale

In this research, studies using elastomeric separators or bands (26), maxillary orthodontic expansion (27, 28), invisible removal aligners (29, 30) or agenesis cases (31) were excluded because the forces used and the perception of pain could be very different from a fixed orthodontic treatment. On the contrary, all studies of patients with each fixed orthodontic treatment have been included.

In one of these studies, patients treated by straight-wire technique with Equilibrium brackets (Dentaurum, Ispringen, Ger many) or with In-Ovation C (GAC/Dentsply, Tokyo, Japan) self-ligating brackets (32) were compared. The results show that there is not a significant difference in average pain between bracket groups during the first week of active orthodontic treatment ($p > 0.05$) (33).

The level of dental crowding of treated patients was also not the same. Some patients had slight crowding (34) or levels up to 5 mm (33). Other subjects had 3-5 mm maxillary dental crowding (21, 35).

In the study of Lo Giudice et al., 90 subjects were divided into three groups with different crowding: mild (3-5 mm), moderate (5-7 mm), and severe (>7 mm). The authors did not find differences in the pain perceived among examined patients with mild, moderate and severe mandibular anterior crowding. However, there is no specific indication for the usage of PBM according to the amount of crowding (36).

However, in some treatments, the patients were subjected to bilateral extraction of the first upper premolars and retraction of the canines to correct protrusion and dental crowding; this means that greater force was used to get more displacement of some teeth. In addition, banding and the Nance button were used to obtain good posterior anchorage transpalatal bars (35,37-44).

Laser procedures

In most studies, the procedure was carried out in an isolated room, using protective glasses for the operator, patient and dental assistant (39). In order to confuse the patient and allow the placebo effect, the non-irradiated side was treated in the same way but with the machine turned off. To prevent the perception of the beeping emitted by the laser, music was played at a high volume (39, 41). In this way, patients could not distinguish between the placebo and experimental sides (37).

An article indicates a beneficial effect even on the side not treated with lasers, indicating a generalised effect within the trigeminal system. However, there have been no effects on extra-trigeminal sensitivity. The authors hypothesise that PBM may have reduced peripheral sensitisation of Aδ fibres and C-related nerve fibres (34).

One of the effects of laser therapy with split-mouth is the probability of carry-across effects of the laser beam from one side to the other (45). Therefore, many authors used a plastic shield like a barrier at the midline to limit the laser beam's penetration and, perchance, alter the results (39, 41).

The lasers used had different wavelengths and power. In addition, the irradiated dosimetry, energy density, timing, points on each side and number of monthly applications were also not the same. For example, in one of these studies, patients were first subjected to the alignment and levelling stages with nickel-titanium archwires, and then when the canine retraction began, with 0.018-in-stainless steel wires, laser therapy was used (37).

In the Domìnguez and Velàsquez study, laser treatment was carried out during the final stage of orthodontic treatment, when stainless steel archwires 0.019x0.025 inch were used (33). These results, in addition to the other studies, make us think that PBM is effective in modulating painful sensation at all stages of orthodontic treatment, or a 3-week low-laser therapy model can be convenient in clinical practice as it coincides with conventional orthodontic appointments (39).

Dosages and ways of energy distribution

Low-level laser therapy usually uses the following parameters: a power density between 5 and 150 mW x cm-2, red and NIR wavelength range of 600-1000 nanometers, applied for 30 to 60s per point. The resulting therapeutic effect depends on energy density measured in joules (J) per cm2 (46, 47).

The effects of PBM depend upon the different tissues, cell type, irradiation parameters, time of exposure and redox state of the cell (48). There is a biphasic dose response which underlines the existence of optimal irradiation and dose parameters. In order to make laser therapy effective, the parameters need to be within the biostimulatory dose windows (49).

It is essential to remember that a higher dosage than the optimal can have negative therapeutic outcomes; on the contrary, a lower dosage than the optimal value might have a diminished effect (50). For the success of the treatment

wavelength and energy (in J), energy density (J/cm2) is necessary, but also the original power, power density and duration parameters (49, 51).

In the studies examined, the wavelength is between 632 and 980 nm, energy varies between 0.7 and 400 mW, and total energy is not indicated in all studies. In addition, all studies indicating the amount of energy are within the efficacy window. These different protocols make it difficult to compare and quantify the beneficial effects on patients (52).

Statistically significant results

In most cases, the different studies compared patients whose mouth was divided into a part treated with laser therapy and a placebo part. The results show a statistically significant difference in perceived pain between the irradiated and non-irradiated arch (33, 34,41, 44).

In their study, Sobouti et al. contributed about a 12.1% reduction of a painful sensation on the laser side compared with the matched placebo side (37).

Another study shows that the irradiated side significantly reduced the average range of dental pain at 3, 7, and 14 days after laser treatment (38, 42). In the study of Dominguez et al., results show that the highest pain intensity occurs in the first 48 h on the treated side (40).

In a study by Alam et al., all patients are randomly divided into 4 groups: PBM + self-ligating bracket, PBM + conventional bracket, non-PBM + self-ligating bracket, and non-PBM + conventional bracket function. Authors revealed PBM + self-ligating results as the best and PBM + conventional as the 2nd best in lessened pain perception (53).

Another study found a statistically significant difference between the placebo/control and irradiated groups. In the first case, the peak of pain appeared on the second day ending around days 6-7. In the second case, the peak of pain came after 6 hours and disappeared on day 4; patients then found a reduced duration of pain (36).

In three studies, the results do not show a statistically significant difference in relieving orthodontic pain sensation following laser therapy (21, 43, 35).

In a study by AlSyed et al., however, the mean pain scores found in the laser group were less than those of the placebo group in all studied time points; this indicates some clinical efficiency of LLL despite the absence of statistical significance (35).

Appearance of pain

Articles used for this research agree that the onset of pain occurred 2-4 hours after the archwire insertion was activated, up to a peak at 24 hours. The painful sensation decreased and disappeared within 7 days (21, 33, 34, 39, 41), in accordance with Koritsanszky et al. (54).

Age and sex difference

It is known that pain perception can be affected by different individual parameters, such as age, sex, pain threshold, the magnitude of the applied force, emotional status, cultural differences, and previous pain experiences (55, 56, 57).

In several studies, however, no significant difference was found in the pain sensation between males and females, nor between adolescents and adults (39, 41).

The inclusion of both genders and different ages favours generalizability, but it is also important to remember that the most sensitive age might be between 13 and 16 years old (6). In these split-mouth designs, each patient was matched with himself/herself so that variations in the subject's demographics did not confuse the results (37).

Different methods of measuring pain

The recording of the painful sensation was done with different parameters. Some studies have used the Visual Analogue Scale (VAS). It is a widely accepted method for measuring and showing differences in pain reported by patients; it is reliable, accepted by patients, sensitive, and reproducible. Although it is a subjective method, it is one of the best methods because of its reliability in scoring pain at different time points when a significant difference among participants is expected (58, 59).

Other articles used a questionnaire based on a numeric rating scale (NRS) of evaluation to investigate the effects of laser therapy on pain sensation. It is highly correlated with VAS (60). This choice also allowed younger patients to comprehend the data collection method (61). Additionally, NRS can be administered verbally during a phone call (62).

Type of machinery and employee operator

Often the method of administration of laser therapy is unclear but, most importantly, not reproducible. In many studies, the protocol involves using the device at different points of the mouth and for a variable period. To increase the method's reliability, many authors had orthodontic treatment and laser applications performed by the same operator (21, 33, 34, 36, 38, 43).

Unfortunately, even the individual operator cannot reproduce his work similarly over time. Therefore, the handpiece is not easily used in a repeatable way at each session (Fig. 1).

In a recent study by Lo Giudice in 2020, ATP38 was used. Depending on the therapeutic indication, the device is equipped with a multi-panel system and a combination of wavelengths ranging from 450 to 835 nm. One of the advantages of using a static device is that the session is independent of the operator; this can enhance the standardisation of the dosage administered since the operator error is eliminated, and it can make the effect reproducible (Fig. 2) (63).

Fig. 1. *It is difficult to use the hand-piece in a repeatable way at each session.*

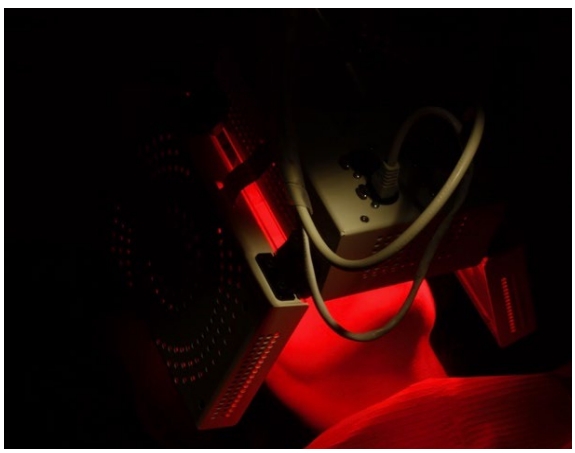

Fig. 2. *ATP38 in use.*

CONCLUSION

This search shows that most authors observed that pain reduction could not be attributed to placebo-based mechanisms. Instead, they said that laser therapy effectively reduces painful sensations during different stages of orthodontic treatment. Other authors showed no statistically significant results in favour of photobiomodulation, but it is important to remember that they used different parameters, including technical specifications and application modes. In this regard, even just one parameter can influence the effect of PBM. Additionally, results depend also on the participants' variability.

To obtain generally valid studies with consistent and reproducible results, it is necessary to standardise the different parameters that are independent of the operator performing the procedure.

Hopefully, suggesting the spread of devices similar to ATP38, the scientific validity of PBM research in orthodontia will increase.

Author Contributions

PC and GC designed the research study; IC performed the research; PC and GC wrote the manuscript. All authors contributed to editorial changes and approved the final version. The authors declare no conflict of interest.

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European Journal of Musculoskeletal Diseases
ISSN 2038-4106/2021

Retrospective Observational Study

RELATIONSHIP BETWEEN MISSING TEETH AND CLEFT SIDE IN NON-SYNDROMIC CLEFT PATIENTS

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ABSTRACT

The purpose of this retrospective observational study was to evaluate the possible association between the missing teeth and the side associated with the cleft in non-syndromic patients. This study consisted of 201 cleft patients including 131 males with a mean age of 12.3 ± 4 years and 70 females with a mean age of 12.6 ± 3.9 years. 148 of the patients were affected by cleft lip and palate, while the other 53 presented only cleft lip. Charts, models, radiographs, and intraoral photographs were used for the study. T-test and chi-square tests were used for the assessment of the data. Hypodontia was found in 129 individuals (64.1%). Chi-square test showed that there was no statistically significant difference between the number of male and female patients with hypodontia (P<0.7). 122 of the patients with hypodontia (60% of the total 201 subjects) had missing maxillary incisors. Totally there were 197 teeth absent in the entire cleft samples. 180 (91.3%) of these teeth were missing on the cleft side and 17 (8.7%) of them were missing on the non-cleft side. In addition, 170 (86.3%) of them were maxillary permanent

Received: 24 May, 2021
Accepted: 28 July, 2021

ISSN: 2038-4106

lateral incisors and only 27 (13.7%) of them were permanent second premolars. The frequency of missing maxillary lateral incisors in cleft lip patients was significantly higher compared with the missing second premolars in both arches. The incidence of lateral incisor missing is significantly higher on the cleft side.

KEYWORDS: *cleft lip, cleft palate, hypodontia, missing, cleft side*

INTRODUCTION

Cleft lip and/or palate (CLP) is one of the most common types of craniofacial birth defects (1). The overall prevalence rate for live births with cleft lip, cleft palate, or both has been reported at 1.39 per 1000 live births (2); it accounts for 65% of all head and neck anomalies (3). Prevalence of dental anomalies such as variations in tooth number and position and reduced tooth dimensions have always been found to be higher in CLP patients than in the whole population (4-10). Akcam et al., 2011 detected that cleft patients had at least one dental anomaly in 96.7 percent of examined subjects and many patients showed other dental and skeletal malocclusion in addition to the cleft lip and palate anomalies.

Shapira et al. (12) reported the most significant number of developmental dental abnormalities in upper lateral incisors in the cleft area, both in deciduous and permanent dentitions. Moreover, in CLP patients are frequent shape anomalies, such as enamel hypoplasia and conoid shape (13, 14). Tooth agenesis also called hypodontia or congenital absence of teeth, is the most detected developmental dental anomaly in all the cleft types (15). Furthermore, Shapira et al. (12) discovered a prevalence of 77% of hypodontia in a sample of cleft patients. Jiroutova and Mullerova (16), about the hypodontia frequency in CLP patients, found that the maxillary arch was involved more frequently in patients with this defect. The dental bud of the upper lateral incisor was often affected in both CL and CLP, while the second lower premolar was most frequently absent in the isolated cleft palate. Paranaiba et al. (17) found that in Brazilian patients with non-syndromic cleft lip and/or palate, the prevalence of agenesis of the premolars and maxillary lateral incisors is higher in unilateral complete cleft lip and palate patients. In many studies, lateral incisors are the most frequent agenesis tooth, followed by second premolars (18, 19). Whereas, in Laatikainen et al. (20) and Ranta et al. (21), the most frequently missing tooth was the upper second premolar, and to follow the maxillary lateral incisor and the lower second premolar.

However, the emerging literature evidence has always been limited to describing numbers and shape anomalies in patients with cleft. In addition, an association between anomalies with the side involved in the cleft and correlating the level of anomalies with those of the cleft has been verified only in few reports.

Considering the discrepancies in the literature, the aim of the current study was to determine the frequency of missing second premolars and lateral incisors in cleft lip/palate patients and compare it with other subjects' data to determine the possible association between the cleft side and the agenesis side.

MATERIALS AND METHODS

The study was carried out under the provisions established by the Declaration of Helsinki. Ethical approval and informed consent were obtained from each subject and a parent or trustee. 201 non-syndromic cleft lip and/or palate patients were included in the study. The patients' population was racially and ethnically similar, and all their parents were of Persian origin.

Exclusion criteria were: cleft patients with craniofacial syndrome; patients with unclear radiographs. Therefore, 201 subjects were enrolled in the study (131 males aged 12.3±4 years and 70 females aged 12.6 ± 3.9 years). The gender distribution of the sample can be observed in Table I.

Table I. *Gender distribution of samples.*

Gender	N (%)	Age (year)
Male	131 (65.2)	12.3 ± 4
Female	70 (34.8)	12.6 ± 3.9

The patients were classified based on Whitaker et al. (22) classification in which the patients were divided into four groups (lip, cleft palate, cleft lip and palate, and cleft lip and alveolus), and each group was divided into two subgroups unilateral or bilateral. Dental casts, orthopantomography, and/or periapical and occlusal X-rays of the patients were used to diagnose possible agenesis (leaving out the wisdom teeth).

A thorough examination of hypodontia of permanent teeth (excluding third molars) was undertaken using panoramic, periapical, and occlusal radiographs. In addition, data regarding missing teeth inside or outside the cleft area were collected, and two observers evaluated the records simultaneously. Their outcomes were blinded to each other. Inter-observer accordance was estimated using kappa analysis. A kappa value of 1 showed perfect agreement. The Statistical Package for Social Sciences, Version 20 (SPSS Inc. Chicago, Illinois, USA) was used to examine the data. The Chi-square test was used to analyze the data, and the p-value was set at $P < 0.05$. During this research, all operators wore surgical masks to prevent the respiratory system virus (23) and to maintain office hygiene (24, 25).

RESULTS

The distribution of samples depending on the type of cleft is shown in Table II. The samples were divided into the unilateral and bilateral cleft lip, cleft lip and palate, cleft lip and alveolus, and cleft palate groups; permanent teeth agenesis was evaluated in every group. Hypodontia was found in 129 patients (64.1%) of the total sample, including 83 boys (41.3%) and 46 girls (22.8%).

Chi-square test highlighted no statistically significant difference between males and females. ($P<0.7$) (Table II) Out of 197 teeth absent in the entire cleft sample (Table III), 180 (91.3%) teeth were missing on the cleft side, and 17 (8.7%) teeth were missing on the non-cleft side. Of these, 170 (86.3%) were upper permanent lateral incisors (160 in the cleft area and 10 in the non-cleft area), and 27 (13.7%) were permanent second premolars (20 on the cleft side and 7 on the non-cleft side) (Table IV). Statistically significant differences were detected between the lateral incisors agenesis in the cleft and non-cleft areas ($P<0.001$).

Table II. *Distribution of samples according to cleft type.*

Gender	UCL	BCL	UCL and alveolus	BCL and alveolus	CP	UCLP	BCLP	Total
Male	1	1	18	8	2	64	37	131
Female	-	1	11	4	7	27	20	70
Total	1	2	29	12	9	91	57	201

*Unilateral cleft lip: **UCL**; Bilateral cleft lip: **BCL**; Cleft palate: **CP**; Unilateral cleft lip and palate: **UCLP**; Bilateral cleft lip and palate: **BCLP***

Table III. *Number of patients with hypodontia according to sex.*

Gender	No. of Patients	Patients With Hypodontia	Patients Without Hypodontia	P Value
Male	131 (65.2%)	83 (41.3%)	48 (23.9%)	0.7
Female	70 (34.8%)	46 (22.8%)	24 (12%)	
Total	201 (100%)	129 (64.1%)	72 (35.9%)	

Table V shows that 122 (60%) of the patients had missing maxillary lateral incisors, which were significantly higher than missing either maxillary or mandibular second premolars. As can be seen, 22 of the total 41 cleft lip and alveolus patients and 95 of the total 148 cleft lip and palate patients had missing laterals, while only 1 of 18 patients had missing second premolars. Table VI shows that all the patients were missing a total of 197 upper lateral incisors and upper and lower second premolars.

Table IV. *Number of missing teeth according to cleft side and non-cleft side.*

	Missing lateral incisor	**Missing second premolars**			**Total missing teeth**
	Maxilla N (%)	Maxilla N (%)	Mandible N (%)	Total N (%)	N (%)
Cleft side	160 (81.2)	14 (7.1)	6 (3)	20 (10.1)	180 (91.3)
Non-cleft side	10 (5.1)	6 (3)	1 (0.6)	7 (3.6)	17 (8.7)
Total	170 (86.3)	20 (10.1)	7 (3.6)	27 (13.7)	197 (100)

Table V. *Number of patients with missing maxillary incisors and missing second premolars according to cleft type.*

	Patients	**Patients with missing Max Lateral Incisors**				**Patients with missing Max/Mand II Premolars**			
		L	R	B	T	L	R	B	T
Cleft lip (CL)	3	0	0	2	2	0	0	0	0
Cleft palate (CP)	9	2	0	1	3	0	0	1	2
CL and alveolus	41	10	6	6	22	0	0	0	1
Cleft lip palate (CLP)	148	30	21	44	95	4	6	8	18
Total	201	42	27	53	122	4	6	9	21

***L**=Left side, **R**=Right side, **B**=Both Sides, **T**=Total number of missing teeth*

Table VI. *Distribution of hypodontia in cleft patients according to number of missing teeth.*

	Max Lateral Incisors				**Max II Premolars**				**Mand II Premolars**			
	L	R	B	T	L	R	B	T	L	R	B	T
Cleft lip (CL)	0	0	4	4	0	0	0	0	0	0	0	0
Cleft palate (CP)	1	0	2	3	0	0	4	4	0	0	2	2
CL and alveolus	10	6	13	29	0	1	0	1	0	0	0	0
Cleft lip palate (CLP)	29	21	84	134	2	3	10	15	0	1	4	5
Total	40	27	103	170	2	4	14	20	0	1	6	7

***L**=Left side, **R**=Right side, **B**=Both Sides, **T**=Total number of missing teeth*

DISCUSSION

This study found that 129 (64.1%) of all cleft patients suffered from hypodontia. These findings correspond to the findings of Shapira et al. (12), who detect the prevalence of 77% hypodontia in their study group of subjects with cleft lip and palate, isolated or associated. These patients were missing one hundred ninety-seven upper lateral incisors and upper and lower premolars. In this study, from a total of 201 patients, 60 % of them had missing maxillary lateral incisors, similar to Suzuki et al. (26), who reported that 56.9% of their cleft lip and/or palate subjects had missing maxillary lateral incisors. Polder et al. (27), about the prevalence of permanent missing teeth in the Caucasian populations of North America, Australia, and Europe, reported that the mandibular second premolar was the most involved too, followed by the maxillary lateral incisor and the maxillary second premolar.

In the current study, 11.5% of second premolars were found to be missing, which is similar to the 18% found by Shapira et al. (12). In our study, the missing second premolars were substantially higher in the maxillary arch in all groups, with a total of 20 missing second premolars in the maxilla and 7 missing in the mandible. These numbers also correspond to the findings of Shapira et al. (28), who reported a 47-second premolars agenesis in the upper arch and agenesis in the lower arch. Nevertheless, the findings of the current study are in contrast with the findings of Laatikainen et al. (20) and Ranta et al. (21). They found that maxillary second premolars were the most frequently absent teeth, followed in order of frequency by the maxillary lateral incisors and the mandibular second premolars in cleft patients. In the current study, the absence of teeth was more frequent on the cleft side, respecting the healthy side, which agrees with the outcomes of Shapira et al. (28).

Ranta et al., in their review, showed that the prevalence of missing teeth grows according to the severity of the cleft (21). This outcome agrees with our work in which there was a high prevalence of teeth agenesis in cleft lip and palate patients and a lower prevalence in isolated cleft lip and isolated cleft palate patients. Moreover, Paranaiba et al. (17) indicated that dental abnormalities were more frequent in unilateral cleft lip and palate subjects compared with bilateral cleft lip and palate subjects. It is also reported that the majority of cleft lip and or palate patients had at least one dental anomaly, and most of the dental anomalies were observed at the side of the cleft. However, no association could be found between the type of cleft and dental anomalies (27).

Furthermore, ethnicity plays a significant part in the prevalence of cleft and associated abnormalities. Polder et al. stated that missing teeth were more frequent in Europe and Australia compared with North America (27). They also showed that the prevalence of missing teeth in females is 1.37 times higher than in males for all three continents. One of the limitations of the current study, which could affect its outcomes, is the small sample size. Moreover, associated dental disturbances and medical pathologies should be explicitly investigated in this type of frailty patient.

More multi-center works with a larger study group and different breeds are needed. In addition, future multidisciplinary studies about the genetics of cleft subjects to confirm the higher prevalence of left-sided is required.

CONCLUSION

In this study, the maxillary lateral incisor agenesis in cleft lip patients (60%) was significantly more frequent than the second premolars agenesis in both arches (11.5%). The prevalence of missing lateral incisors raises definitely according to the severity of the cleft and is significantly higher on the cleft side.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

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European Journal of Musculoskeletal Diseases
ISSN 2038-4106/2021

Evaluation Study

THE EFFECT OF HYALURONIC ACID ON HUMAN FIBROBLASTS: AN IN VITRO STUDY

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ABSTRACT

The linear glycosaminoglycan hyaluronic acid is a component of many body organs and tissues, such as the extracellular matrix of connective tissue, skin, synovial fluid, and embryonic mesenchymal tissue, naturally occurring with a high molecular weight. Hyaluronic acid can also be detected in the soft periodontal tissues (gingiva and periodontal ligament).

Hyaluronic acid biocompatibility, biodegradability, non-toxicity, non-immunogenic and non-inflammatory properties make it applicable to bioengineering and biomedicine fields. Its molecular weight influences the biological effects of hyaluronic acid.

High-molecular weight hyaluronic acid causes the suppression of immune response, avoiding the exacerbations of inflammation; on the other hand, low-molecular-weight hyaluronic acid takes part in tissue damage signaling and immune cell mobilization. Thanks to these properties, hyaluronic acid can be considered a promoter of soft tissue and bone healing.

This study evaluates the effect of hyaluronic acid with different molecular weights on fibroblasts.

KEYWORDS: *hyaluronic acid, inflammation, fibroblasts*

INTRODUCTION

The linear glycosaminoglycan hyaluronic acid (HA) is composed of repeating units of N-acetylglucosamine and a linear chain of D-glucuronic acid, with β-1,3 and β-1,4 glycosidic bonds linking the monosaccharide units (1-4). HA is naturally occurring with a high molecular weight (up to $2x10^4$ kDa) and is a component of extracellular matrix of connective tissue, skin, synovial fluid, and embryonic mesenchymal tissue (5, 6). Regarding the oral cavity, HA can be detected in the soft periodontal tissues (gingiva and periodontal ligament) and hard tissues such as bone; moreover, it represents a key element

Received: 23 June 2021
Accepted: 17 August 2021

ISSN: 2038-4106

of crevicular fluid and saliva (7, 8). This natural linear polysaccharide is synthesized by three transmembrane enzymes 1, 2, and 3 (HAS1, 2, and 3) on the cytoplasmic surface of the plasma membrane, while the hyaluronidase enzyme family plays a major role in its catabolism (9). The HAS synthesized HA with different molecular weights since their expression during morphogenesis, and pathological conditions are different: HAS1 and HAS2 are responsible for the production of high-molecular sized molecules (> 2 x 10^6), while low-molecular-weight molecules are generated by HAS3 (1 x 10^5 – 1 x 10^6) (10-13). HA biocompatibility, biodegradability, non-toxicity, non-immunogenic and non-inflammatory properties make it applicable to bioengineering and biomedicine fields (14). Its molecular weight influences the biological effects of HA: HA with a high-molecular size (> 500 kDa) acts as an anti-angiogenic, anti-inflammatory, and immunologic depressant molecule, and it can enhance tissue integrity and cell quiescence (9, 15). Molecular weight from 6 to 20 kDa presents angiogenic, immunostimulatory, and phlogotic properties; embryonic development, ovulation, and wound healing involve HA with molecular weight ranging from 20 to 200 kDa (9).

Data reported in the literature demonstrates that HA can contribute to the oral soft tissue wound healing process: it first acts as a high molecular weight molecule whose anti-angiogenic and immunosuppressive properties facilitate the accumulation of polymorphonuclear leukocytes in the wound site. In the next step, the inflammatory stage, high molecular weight HA is fragmented by hyaluronidase or reactive oxygen species activity, inducing angiogenesis, stimulating the production of pro-inflammatory cytokines by fibroblasts, cementoblasts, and osteoblasts and thus promoting the inflammatory response (16-18). It has been demonstrated that high-molecular-weight HA causes the suppression of immune response, avoiding the exacerbations of inflammation, while on the other hand, low-molecular-weight HA takes part in tissue damage signaling and immune cell mobilization (19). Thanks to these properties, HA can be considered a valid promoter of soft tissues and bone healing (20).

The aim of our research was to study the effect of HA with different molecular weights on human fibroblasts, assessing the role of this natural linear polysaccharide in gingival inflammatory conditions.

MATERIALS AND METHODS

Primary Human Fibroblast cells culture

Primary gingival fibroblasts obtained from a 60-year-old woman were purchased from ATCC® Cell Lines (LGC Standards, Middlesex, UK). Cryopreserved cells at the second passage were cultured in 75 cm^2 culture flasks containing DMEM medium (Sigma Aldrich, Inc., St Louis, Mo, USA) supplemented with 50% fetal calf serum, antibiotics (Penicillin 100U/ml and Streptomycin 100 micrograms/ml-Sigma Aldrich, Inc., St Louis, Mo, USA).

Cells were incubated in a humified atmosphere of 5% CO_2 at 37°C. The medium was changed the next day and twice a week. After 15 days, the pieces of tissue were removed from the culture flask. Cells were harvested ted after an additional 24h of incubation.

Cell viability test

PrestoBlue™ Reagent Protocol (Invitrogen) was used to evaluate the viability of cells treated with high, low, and medium molecular weight hyaluronic acid (HA) solutions at different concentrations. A 10 g/mL stock solution of each molecular weight HA was prepared. Further dilutions were made with the culture medium to the desired concentrations before use. Serial dilutions of each different molecular weight HA solution (1000 mg/mL, 100 mg/mL, 10 mg/mL, 1 mg/mL) were added (three wells for each concentration). The cell culture medium alone was used as a negative control. Cells were seeded into 96-well plates at a density of 104 cells per well containing 100 µl of cell culture medium.

After 24h of incubation, cell viability was measured using PrestoBlue™ reagent protocol (Invitrogen). The percentage of viable cells was determined by comparing the average absorbance in drug-treated wells with the average absorbance in control wells exposed to vehicles alone. The results were presented as the mean ± standard deviation of three measures.

Cell treatment

Cells were seeded at a 1.0 x 10^5 cells/ml density into 9 cm^2 (3 ml) wells and subjected to serum starvation for 16 hours at 37°C.

After serum starvation, cells were treated with the following solution: a) 10 mg/mL of high molecular weight HA; b) 10 mg/mL of medium molecular weight HA; c) 10 mg/mL of low molecular weight HA.

All solutions were obtained in DMEM supplemented with 2% FBS, antibiotics, and amino acids.

For each treatment, three biological replicates were performed.

The cells were maintained in a humidified atmosphere of 5% CO^2 at 37 °C for 24 h.

Cell medium alone was used as a negative control.

After the end of the exposure time, cells were trypsinized and processed for RNA extraction.

RNA isolation, reverse transcription, and quantitative real-time RT-PCR

Total RNA was isolated from cell lines using GenElute mammalian total RNA purification miniprep kit (Sigma-Aldrich) according to the manufacturer's instructions. Pure RNA was quantified at NanoDrop 2000 spectrophotometer (Thermo Scientific).

cDNA synthesis was performed starting from 500 ng of total RNA, using PrimeScript RT Master Mix (Takara Bio Inc.). The reaction was incubated at 37°C for 15 min and inactivated by heating at 70°C for 10 sec. cDNA was amplified by Real-Time Quantitative PCR using the ABI PRISM 7500 (Applied Biosystems).

All PCR reactions were performed in a 20 µl volume. Each reaction contained 10 µL of 2x qPCRBIO SYGreen Mix Lo-ROX (Pcrbiosystems), 400 nM concentration of each primer, and cDNA.

Custom primers belonging to the "Inflammatory Cytokines and Receptors" pathway were purchased from Sigma Aldrich. The selected genes grouped by functional pathway are listed in Table I.

All experiments were performed, including non-template controls, to exclude reagent contamination. PCR was performed, including two analytical replicates.

The amplification profile was initiated by 10 A minute incubation at 95°C, followed by two-step amplification of 15 seconds at 95°C and 60 seconds at 60°C for 40 cycles. As a final step, a melt curve dissociation analysis was performed.

Table I. *Selected genes used in Real Time PCR grouped by functional pathway.*

Pathway	Gene symbol	Gene name
Chemokine	CCL2	C-C motif chemokine ligand 2
	CCL5	C-C motif chemokine ligand 5
	CCL8	C-C motif chemokine ligand 8
	CXCL5	C-X-C motif chemokine ligand 5
	CXCL10	C-X-C motif chemokine ligand 10
Chemokine receptor	CCR1	C-C motif chemokine receptor 1
	CCR2	C-C motif chemokine receptor 2
	CCR5	C-C motif chemokine receptor 5
	CCR6	C-C motif chemokine receptor 6
	CCR10	C-C motif chemokine receptor 10
	CXCR5	C-X-C motif chemokine receptor 5
Interleukin	IL1A	interleukin 1 alpha
	IL1B	interleukin 1 beta
	IL2	interleukin 2
	IL3	interleukin 3
	IL5	interleukin 5
	IL6	interleukin 6
	IL7	interleukin 7
	IL8	interleukin 8
Interleukin receptor	ILR1	interleukin 1 receptor type 1
	IL1RN	interleukin 1 receptor antagonist
	IL6R	interleukin 6 receptor
	IL10RB	interleukin 10 receptor subunit beta
Cytokine	BMP2	bone morphogenetic protein 2
	SPP1	secreted phosphoprotein 1
	TNFSF10	TNF superfamily member 10
	TNFSF11	TNF superfamily member 11
	VEGFA	vascular endothelial growth factor A
Cytokine receptor	TNFRSF	tumor necrosis factor receptor superfamily
Housekeeping gene	RPL13	ribosomal protein L13

Statistical analysis

The gene expression levels were normalized to the expression of the reference gene (RPL13) and were expressed as fold changes relative to the expression of the untreated cells. Quantification was done with the delta/delta Ct calculation method (21).

RESULTS

To establish the right concentration of hyaluronic acid (high, medium, and low molecular weight) to be used in treating fibroblasts cultured in vitro, serial dilutions of the stock solutions were made. After treating the cells for 24 hours with these solutions, cell viability was measured using the PrestoBlue™ assay, and it was established that the optimal concentration of the treatment that did not significantly affect cell viability was 10 mg/ml for all three types of hyaluronic acid.

Gene expression of genes belonging to the "Inflammatory Cytokines and Receptors" pathway was investigated in fibroblast treated high, medium, and low molecular weight hyaluronic acid solution 10 mg/ml for 24 h. Table II shows significant gene expression levels after 24h treatment with high molecular weight hyaluronic acid (HMW-HA) compared to untreated cells.

The treatment of fibroblasts with HMW-HA modulates the expression of various genes belonging to the "Inflammatory Cytokines and Receptors" pathway. Among these, the up-regulated genes are the chemokines CCL2D and CCL8 and the receptor for the chemokines CXCR5. In addition, the treatment induces a significant up-regulation of interleukins IL1B, IL2, and IL5 and the over-expression of a cytokine that belongs to the tumor necrosis factor (TNF) ligand family, TNFSF10.

Among the genes down-regulated by HMW-HA are the CCR1 chemokine receptor, interleukin 1 A (IL1A) and its ILR1 receptor, and the two cytokines BMP2 and SPP1. Fig. 1 represents the gene expression profile of treated fibroblast with HMW-HA compared with control (untreated cells).

Table III reports the significant gene expression levels after a 24h treatment with medium molecular weight hyaluronic acid (MMW-HA) compared to untreated cells. Significant up-regulated genes were chemokine receptors CCR2 and CCR10, the interleukin 2 (IL2), and the cytokine belonging to the tumor necrosis factor (TNF) ligand family, TNFSF11. Conversely, MMW-HA induces down-regulation of genes like the chemokine receptor CCR6, the interleukin 6 (IL6), the receptor IL6R, and the cytokine SPP1.

Fig. 2 shows the expression profile of genes up-and down-regulated in treated with medium molecular weight hyaluronic acid.

Table IV reported the significant gene expression levels after a 24h treatment with low molecular weight hyaluronic acid (LMW-HA) compared with untreated cells. The treatment induces the down-regulation of genes such as chemokine CCL5 and chemokine receptors CCR1, CCR5, CCR6, and CCR10. Other down-expressed genes were Interleukin IL1A, the interleukin receptor IL6R and the cytokine SPP1.

Only the interleukin IL2 and the cytokine belonging to the tumor necrosis factor (TNF) ligand family, TNFSF11, were up-regulated by the treatment. Fig. 3 shows the fibroblast gene expression modulation after the LMW-HA treatment.

DISCUSSION

Based on its characteristics, such as its

Table II. *Significant gene expression levels after 24h treatment with HMW-HA, as compared with untreated cells.*

Gene	Fold change	SD +/-)	Gene function
CCL2D	2,30	0,34	Chemokine
CCL8	3,79	0,11	Chemokine
CCR1	0,28	0,04	Chemokine receptor
CXCR5	2,49	0,87	Chemokine receptor
IL1A	0,34	0,00	Interleukin
IL1B	2,83	0,03	Interleukin
IL2	7,35	0,15	Interleukin
IL5	2,94	0,01	Interleukin
ILR1	0,41	0,02	Interleukin receptor
BMP2	0,06	0,01	Cytokine
SPP1	0,31	0,01	Cytokine
TNFSF10	5,12	1,19	Cytokine

Table III. *Significant gene expression levels after 24h treatment with MMW-HA, as compared with untreated cells.*

Gene	Fold change	SD (+/-)	Gene function
CCR2	3,56	0,14	Chemokine receptor
CCR6	0,28	0,02	Chemokine receptor
CCR10	3,06	0,68	Chemokine receptor
IL2	11,12	3,07	Interleukin
IL3	0,31	0,04	Interleukin
IL6R	0,25	0,02	Interleukin receptor
SPP1	0,49	0,03	Cytokine
TNFSF11	4,71	0,09	Cytokine

Table IV. *Significant gene expression levels after 24h treatment with LMW-HA, as compared with untreated cells.*

Gene	Fold change	SD (+/-)	Gene function
CCL5	0,42	0,0312	Chemokine
CCR5	0,09	0,0009	Chemokine receptor
CCR1	0,47	0,1241	Chemokine receptor
CCR10	0,50	0,0385	Chemokine receptor
CCR6	0,26	0,0115	Chemokine receptor
IL2	6,84	0,0931	Interleukin
IL6R	0,30	0,0070	Interleukin receptor
SPP1	0,50	0,1163	Cytokine
TNFSF11	2,27	0,0178	Cytokine

good biocompatibility, biodegradability, and viscoelastic properties, HA is considered an important biomaterial for tissue engineering, drug delivery systems, and various medical and pharmaceutical applications (22, 23).

HA is also known to reduce the appearance of wrinkles and accelerate wound healing. In addition to these functions, HA-based formulations have shown remarkable efficacy in treating a wide range of inflammatory skin diseases (24). In this study, the effects of HA with different molecular weights on human fibroblasts were evaluated, assessing the role of this natural linear polysaccharide in gingival inflammatory conditions.

The main receptor for HA is CD44, a glycoprotein expressed on the T cell's surface. The binding of exogenous HA to CD44 plays multiple roles in T cell biology, including their autocrine proliferation (25) and the regulation of T cell trafficking to inflamed sites (26). Mahaffey et al. showed that exogenous HA enhances IL2 expression, which binds to its IL2R receptor inducing T cell proliferation (27).

In this study, the treatment of fibroblasts with hyaluronic acid at all three molecular weights (high, medium, and low) induces the overexpression of IL2, suggesting that IL2 is an important mediator involved in the proliferation of T lymphocytes following the binding of HA to its CD44 receptor. SPP1 encodes for osteopontin, a protein that plays a fundamental role in the immune response and tissue repair associated with inflammatory diseases. Its expression increases in immune cells, epithelial cells, endothelial cells, and fibroblasts of inflamed tissues.

Mori et al. (28) showed that inflammation-triggered osteopontin expression inhibits the repair rate and contributes to wound fibrosis. In our study, hyaluronic acid determines the under-expression of SPP1. Therefore, hyaluronic acid would increase tissue repair by improving the rate and quality of healing.

In our study, high molecular weight hyaluronic acid exerts an anti-inflammatory effect on treated fibroblasts, as demonstrated by the significant under-expression of IL1 and its IL1R receptor. IL-1 is a pro-inflammatory cytokine whose role in regulating the mechanisms leading to the amplification of inflammation has been widely demonstrated. Its role is primarily expressed in regulating genes that amplify or support inflammation, such as metalloproteinases, prostaglandins, adhesion molecules on leukocytes, and chemokines necessary to recruit circulating leukocytes. Its inflammatory activity has also been extensively demonstrated in vivo in mice, in which it has been observed that the deletion of the gene leads to a reduction of the inflammatory response, exposing them to many

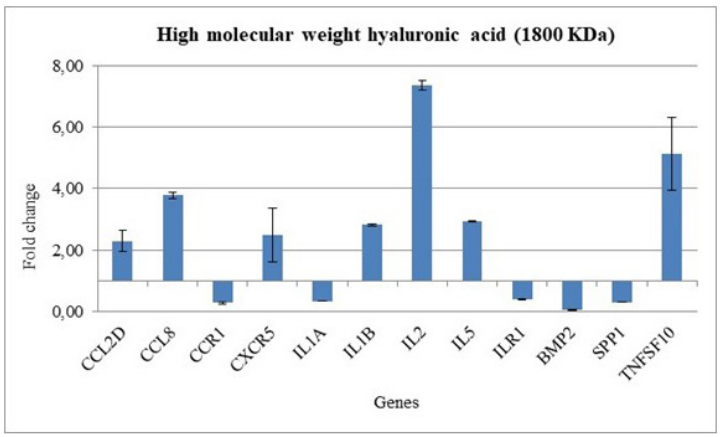

Fig. 1. *Gene expression profile of fibroblast treated with HMW-HA 10 mg/ml.*

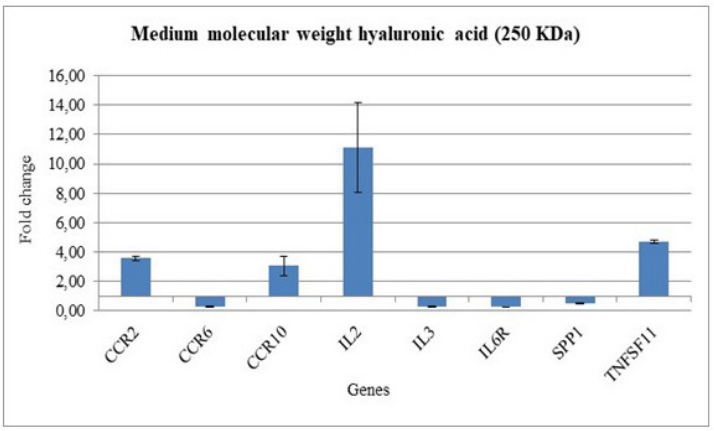

Fig. 2. *Gene expression profile of fibroblast treated with MMW-HA 10 mg/ml.*

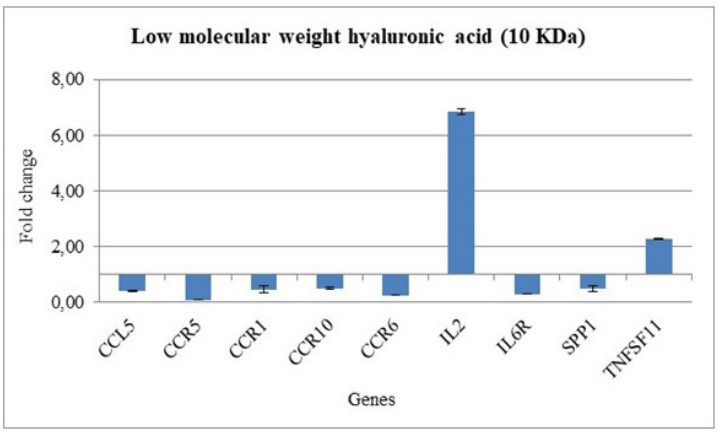

Fig. 3. *Gene expression profile of fibroblast treated with LMW-HA 10 mg/ml.*

types of infections (29).

In general, the results obtained show the tendency of hyaluronic acid to reduce inflammation and tissue regeneration, with a more marked aptitude for high molecular weight hyaluronic acid.

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European Journal of Musculoskeletal Diseases
ISSN 2038-4106/2021

Letter to the Editor

PARRY-ROMBERG SYNDROME – AN UPDATE

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ABSTRACT

An uncommon condition known as Parry Romberg Syndrome (PRS) or Progressive Hemifacial Atrophy typically affects one side of the face and results in the loss of both soft and hard tissues. The illness occurs quickly. Aesthetic and functional losses brought on by the breakdown of soft and hard tissue are exacerbated by the existence of concomitant illnesses such as neuralgia, migraine, epilepsy, and ocular involvement. The age which the disease initially reveals itself determines the severity of the malformation. The severity of the malformation increases with age. Significant psychological stress and social issues are experienced by these patients. The specific cause is unknown, and the majority of the treatment is cosmetic.

KEYWORDS: *Parry-Romberg Syndrome, PRS, Progressive Hemifacial Atrophy*

INTRODUCTION

An uncommon condition known as Parry-Romberg syndrome (PRS) causes atrophy of the skin, subcutaneous fat, muscles, and, in very rare cases, bone structures. Women are more likely to have it. Usually, just one side of the face is involved. It is uncommon for the body to be involved ipsilaterally, and 20% of cases are bilateral. Even though certain cases of PRS have been reported with a late onset, the condition often manifests in the first ten years of life. Over a period of two to twenty years, it gradually advances before stabilising (1).

The atrophic skin could look glossy and hyperpigmented. Linear scleroderma “en coup de sabre” (“strike of the sword”) is a condition in which certain patients have a linear, scar-like depression close to the centre of the forehead. Muscle atrophy, hypoplasia of the underlying bone, and fat loss may cause the face to appear caved in as the condition worsens. Enophthalmos frequently occurs from orbital involvement. Alopecia, epilepsy, facial paresthesia, and trigeminal neuralgia are some more possible side effects. The tongue may have unilateral atrophy intraorally. The maxillary teeth may become visible due to upper lip atrophy. An open bite, inadequate root development, or root resorption may be visible

Received: 10 June 2021
Accepted: 12 August 2021

ISSN: 2038-4106

in teeth on the affected side. Over the course of two to twenty years, progressive hemifacial atrophy gradually worsens until stabilising. Methotrexate, which is frequently coupled with systemic corticosteroids, can be used to treat active disease. The cosmetic defect may be improved with plastic surgery. Additionally, orthodontic treatment is frequently required to correct any underlying malocclusions (2).

Epidemiology

Considering a male-to-female ratio of one to three, the incidence of PRS ranges from 0.3 to 2.5 per 100,000 people each year. Excluding a few cases in the geriatric age range, PRS often begins in the first two decades of life. The average age of onset is 13.2 years, with males being more likely to receive a diagnosis earlier. The illness progresses, then "burns out" after 2 to 20 years and goes into spontaneous remission. Up to 35% of patients have ophthalmic involvement. 15% to 20% of people get neurological symptoms (3).

Biology and pathology

Numerous hypotheses have been put out regarding the cause of hemifacial atrophy, including those involving genetics, trigeminal neuritis, trauma, endocrine issues, viral infection, autoimmune, sympathetic dysfunction, and a connection to a connective tissue illness, particularly scleroderma. (4)

It is still unclear what exactly causes Parry-Romberg syndrome (PRS). This unusual degenerative disease has no known genetic tendency. Romberg's original theory was that atrophic vasomotor neuritis is what causes the illness. However, up to a third of those who are impacted have a history of trauma or surgery. With varying degrees of success, studies have also examined the function of autoantibodies or autoimmune infectious agents. A key factor that has also been hypothesised is a brain abnormality that affects fat metabolism. However, none of the hypotheses hold up to careful examination, and the root cause of hemifacial atrophy is still unknown.

PRS frequently coexists with problems of the nervous system, the heart, the eyes, the joints, the endocrine system, the maxillofacial region, and the teeth. The most frequent association is neurological, which includes migraine, hemiplegia, brain atrophy, and intracranial vascular abnormalities. Because of this, some writers classify the PRS as a "neuro-cutaneous syndrome" (1).

Possible theories to explain biology of PRS

There is ongoing discussion on the exact etiopathogenesis. The following are the theories that are most widely accepted (3):

The "trophoneurosis" theory: The initial theories involved trophic fibre malfunction in the trigeminal and other peripheral nerves. This notion is supported by reports of trigeminal neuralgia and chronic face pain. Confocal microscopy evidence supports this notion by demonstrating a decrease in corneal nerve endings, which are supplied by the ophthalmic division of the trigeminal nerve.

Immune-mediated mechanism: The commonly accepted opinion is that PRS is an autoimmune illness. There has been a lot of overlap with the condition known as linear morphea, a type of systemic sclerosis. In a small number of cases, it has been reported that autoimmune conditions such systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, ankylosing spondylitis, vitiligo, and thyroid abnormalities also exist. Anti-nuclear antibodies offer a clear-cut serological foundation.

Sympathetic dysfunction: Similar to PRS, swelling or dysfunction of the "superior cervical sympathetic plexus" results in enophthalmos, ipsilateral face atrophy, and bone atrophy. Ipsilateral sympathectomy demonstrates a reversal of the disease's course.

Neuro-vasculitis theory: The cerebral lesions' histology shows lymphocytic vasculitis, which resembles Rasmussen encephalitis. Aneurysms and other cerebrovascular abnormalities that are occasionally seen in PRS patients are explained by interstitial neurovasculitis comprising the main arteries. Along the trigeminal nerve's fibers, similar lesions are documented.

Neural crest cell disorder: The existence of soft tissue cancers, cerebral vascular abnormalities, and aneurysms raises the possibility that neural crest cell migration is dysregulated.

Hereditary mechanism: In some cases, PRS is a hereditarily degenerative disorder, however there have only been a

few familial cases reported thus far. No particular gene or inheritance pattern has been discovered, up today.

Trauma-related: Online polls of PRS patients have revealed a contentious link between a brain injury inflicted during infancy and the development of symptoms.

Infectious causes: Risk factors include prior infections with the borrelia burgdorferi, herpes simplex, and varicella zoster viruses.

Pathogenesis and clinical course

Pathogenesis of PRS is not clear (5). The study suggested the possible neurotrophic pathogenesis of PRS since the underlying mechanism of PRS follow the same pattern and pathways of "trigeminal nerve innervation". Some studies also reported the PRS disorder was supposed to be familial one. The anatomical changes resulting from PRS also have a considerable impact on the possible growth potential of hard tissues. These changes halt the increase in the size throughout the growth phase. Those soft tissues who are linked with this also undergo shrinkage process due to adipose tissue loss. This is the reason that facial atrophy which used to happen during second life decade is comparatively less observed because till that time, the facial bone also truly developed. Early onset of PRS and a longer duration result is observable facial deformity.

Most often, the onset PRS happen during the first as well as second decade of life. Women are reported to be affected more than men. Most often PRS has an impact on the left side of face. The characteristics study of PRS showed that this disease develops throughout many years and after a point, it become stable. The condition can also "burn" itself when it is at its starting phase. This will result in somehow minimal facial deformity. Changes regarding the deformity, involved processes and PRS duration can be stabilized during growth. The further extension of facial atrophy is limited since one side of face affected. Also, the involvement of patient's body is ipsilateral.

Clinical findings show that darkly pigmented skin can become dry. Some patients have a line separating their normal skin from their abnormal skin, resembling a large linear scar called a "coup de sabre," as seen in this instance. Enophthalmy, caused by fat loss around the orbit, is perhaps the most frequent symptom of ocular involvement and has been seen. The eye typically functions normally. Localized alopecia may exist. There might occasionally be some neurological side effects such trigeminal neuralgia, facial paresthesia, excruciating headaches, and contralateral epilepsy (4).

Treatment and management

Despite the fact that there is no treatment for this condition, numerous efforts have been made to both halt its progression and rectify any leftover defects. There have been no recorded clinical trials, and it is very difficult to anticipate how a patient will respond to treatment. As a self-limiting condition, Parry Romberg Syndrome calls for medical attention only when it coexists with other autoimmune diseases like scleroderma. In severe and progressing cases of PRS, immunosuppressants like cyclophosphamide, methotrexate, corticosteroids, and azathioprine have been utilised. The standard treatment for active illness is methotrexate (3). The highest weekly dose of methotrexate in oral or injectable form is 25 mg, and the dose varies from 0.3 to 1 mg/kg/week. It is frequently taken in combination with oral prednisolone, 1 mg/kg/day reduced at the conclusion of three months. Surgery to address any remaining deformities constitutes the primary type of treatment for Parry Romberg syndrome. To get a long-lasting outcome, it requires repeated operations (6).

CONCLUSION

For the most part, skin and subcutaneous tissues are affected by PRS, an uncommon, self-limiting, and slowly progressing hemiatrophy of the face that may also affect deeper tissues including the muscles, cartilage, and osseous elements. Although there are numerous neurologic as well as ophthalmologic symptoms, the underlying aetiology is yet unknown. In severe and progressing cases of PRS, immunosuppressants like cyclophosphamide, methotrexate, corticosteroids, and azathioprine have been utilised. Radiologic evaluations can be used to monitor illness development, exclude alternative differential diagnoses, assess post-treatment outcomes, and estimate the degree of the disease.

Conflict of interest

The author declares that there are no conflicts of interest.

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