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# **Original Article**

# **Evaluation of Vision in Gnathological and Orthodontic Patients with Temporomandibular Disorders: A Prospective Experimental Observational Cohort Study**

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Objectives: Temporomandibular disorders (TMDs), orthodontic diseases, and vision dysfunctions seem to be strictly related. The purpose of this study was to prove the relationship, to evaluate the prevalence and the distribution of vision defects in dysfunctional and orthodontic patients, and to establish the type of the relationship. Materials and Methods: A total of 100 patients with TMDs were selected and studied through epidemiological analyses of the following factors: gnathological parameters (temporomandibular joint pathologies according to Diagnostic Criteria for Temporomandibular Disorders); occlusal and skeletal parameters (overjet, overbite, dental class, transversal discrepancies, and mandibular asymmetry); and orthoptic parameters (refractive defects and oculomotor diseases). A prospective experimental observational cohort study was conducted. A comparison with the average frequency of vision defects of the Italian population was performed. The prevalence of vision defects was evaluated. All gnathological and orthodontic parameters were associated with the orthoptic ones. A descriptive and statistical analysis of the data was carried out with the Statistical Package for the Social Sciences software; z test (P < 0.05), frequency analysis (frequency >50%), chi-square test, and Student's t test (P < 0.05) were performed. The scientific consistency was evaluated by using the scientific criteria of Bradford Hill. Results: The comparison with the Italian population showed a higher frequency of refractive defects in the study sample (P < 0.001). The most frequent vision defects were phorias (92%) and tropia (3%). The increased frequency of ocular convergence reduction in the presence of disc displacement with reduction was significant (n = 28; 60%; P < 0.05). In the presence of asymmetry, low frequencies of astigmatism (n = 18; 30%) were observed compared to its absence (n = 22; 54%) (P < 0.05) and high frequencies of motor ocular deviations (n = 59; 100%) were observed compared to its absence (n = 36, 88%) (P < 0.05). In the presence of headache, low frequencies of emmetropia (n = 13; 22%) and higher frequencies of hyperopia (n = 18; 30%) were observed (P < 0.05). Two of five scientific criteria of Bradford Hill were met. Conclusion: It seems to emerge a possible positive relationship between

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TMD and vision defects. In particular, the most interesting associations were found between functional or skeletal orthognathic alterations and oculomotor dysfunctions. However, it was not possible to establish the type of relationship.

**KEYWORDS:** Malocclusion, orthodontics, temporomandibular disorders, vision defects, vision dysfunctions

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### Introduction

ommon comorbidities such as balance alterations, cervical problems, tinnitus, orofacial pain, headache, and emotional disorders are widely discussed in literature. Little interest has been shown, however, in the relationship between temporomandibular disorders (TMDs) and vision disorders, although both are very widespread in the Italian population.[1-3] The two systems, stomatognathic and oculomotor, represent the main exteroceptors of the head-neck district, capable of influencing the balance and behavior of patients.[4] The correlation between the two systems is expressed through three types of connection: anatomical through craniofacial sutures;<sup>[5,6]</sup> neurological through a connection between the encephalic nuclei such as the vestibular, trigeminal, oculomotor, and accessory nuclei:[4,7-11] and functional through the muscle chains.[12,13] Only recent studies, even if not very specific and controversial, have introduced a possible connection between the two systems. Some scientific researches have correlated vision defects, such as astigmatism, hyperopia, myopia, and strabismus, with orthodontic alterations such as first-, second-, and third-class malocclusions and crossbite.[14-20] Monaco et al.[21,22] in two studies of 2003 and 2005 have shown that mandibular deviation and myofascial pain lead to a reduction in ocular convergence, whereas Cuccia and Caradonna<sup>[23]</sup> have shown that binocular vision is reduced in patients with temporomandibular joint disc disorders. There are no studies on many samples that can establish the report with scientific certainty. The correlation between each individual TMD and each individual vision defect is not specified. No scientifically validated classification systems have ever been used to identify the various TMDs. The type of relationship between the two entities (comorbidities and causeeffect) is not yet absolutely clear. The low quality of the works, their heterogeneity, and the difficulty of making valid comparisons require great caution in considering these connections scientifically sustainable, both in diagnostic phase and in the rapeutic phase. To verify the existing report more clearly, an experimental study was carried out with the following four objectives:

- 1. To verify an epidemiological relationship between TMDs and vision defects
- 2. To assess the prevalence of vision defects in gnathological and orthodontic patients
- 3. To analyze the distribution of vision diseases in dysfunctional and orthodontic patients
- 4. To describe the type of relationship between orthognathic and orthoptic diseases

### MATERIALS AND METHODS

A consecutive series of 334 patients from the Units of Gnathology and Orthodontics, Department of Head and Neck, Umberto I Hospital, Sapienza University of Rome, Rome, Italy, was selected from December 2017 to June 2018. Specifically, 231 patients were selected from the Unit of Gnathology and 103 patients from the Unit of Orthodontics. All patients were screened for TMDs by the same calibrated personnel according to the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) of Axis I. A prospective experimental observational cohort study was conducted. All patients had been previously informed about the study, including its aims and the potential risks, and they were given an informed consent form. The study was approved by the Institutional Ethics Committee of Sapienza University of Rome (Approval no. 12/2018 – 0000106). The sample was selected at two levels of evaluation: (1) gnathological one (first assessment) and (2) orthoptic one (second assessment) [Figure 1].

### **FIRST ASSESSMENT**

All 334 patients were analyzed through a complete gnathological specialist examination by the same calibrated personnel by using a specific medical record, developed in accordance with the hospital system. Subsequently, a first clinical form, developed specifically for the study, was filled in with the most relevant data for the experimental study [Figure 2]. All patients with at least one TMD according to the Axis I of DC/TMD classification were included in the study. Instead, patients showing dentoskeletal malformations with asymmetry superior to 4mm, complete removable dentures, fixed prosthesis on more than two lateral-posterior dental elements, absence of more than lateral-posterior dental elements, systemic diseases, and patients who had already



### **GNATHOLOGICAL MEDICAL RECORD**

ID PATIENT (n°)							
PERSONAL DATA							
SURNAME	NAME						
Gender	Birthdate						
Telephone Number			Fiscal Code				
OCCLUSAL PARAMET	ERS						
MALOCCLUSION		I CLAS	SS II CLAS	SS   II CLASS   III CLASS			
OVI		OVB	CROSSB	ITE			
VERTICAL POSTERIOR	R HEIGHT LOSS		ASYMMETRY		*II CLASS: 1	° div. 2° div.	
ALGIC SYMPTOMS (V	'AS scale)		CLINICAL PA	RAMET	TERS		
	DX	sn			RIGHT	LEFT	
JOINT PAIN			CLICKING		-		
MUSCLE PAIN			- CRACKLING		,		
HEADACHE			OTHER NOI	SES	<del>.</del>		
NECK PAIN			LMO				
9			LIG. LAXITY		1		
DC/TMD DISORDERS					PARAFUNCTION	IAL HABITS	
MYALGIA					BRUXISM		
MYOFASCIAL PAIN					CLENCHING		
ARTHRALGIA							
HEADACHE ATTRIBUE	ETED TO TMD						
DISC DISPLACEMENT	WITH REDUCT	ION					
DISC DISPLACEMENT WITH REDUCTION WITH INTERMITTENT LOCKING							
DISC DISPLACEMENT	DISC DISPLACEMENT WITHOUT REDUCTION WITH LIMITED OPENING						
DISC DISPLACEMENT	WITHOUT RED	OUCTION WITH	IOUT LIMITED OPENIN	G 🗌			
DEGENERATIVE JOINT	T DISEASE						
SUBLUXATION							
Department of Oral and Ma Unit of Gnathology Via Caserta 6, 00161 Rome		s				SAPIENZA UNIVERSITÀ DI ROMA	

**Figure 1:** Flowchart of the study

undergone gnathological or orthodontic treatments were excluded. The variables considered were as follows: personal data; occlusal parameters such as malocclusions (presence or absence), overjet and overbite (increased or decreased), and posterior vertical height loss (presence or absence); skeletal parameters such as mandibular asymmetry (presence or absence on radiography); pain

symptoms such as arthralgia, myalgia, neck pain, and headache (scale); and gnathological clinical parameters such as clicking, crackling, other noises, limited mouth opening (LMO), and ligamentous laxity (presence or absence). International DC/TMD such as myalgia, myofascial pain, arthralgia, headache due to TMD, disc displacement with reduction (DDR), DDR

with intermittent locking, disc displacement without reduction (DDNR) with limited opening, DDNR without limited opening, degenerative joint disease, and subluxation (presence or absence) were used for the formulation of the diagnosis.<sup>[24]</sup> The first assessment population comprised 123 patients.

### **S**ECOND ASSESSMENT

The 123 patients previously selected were sent to the visual postural center of the same polyclinic. All patients underwent a second evaluation according to orthoptic exclusion criteria such as the presence of eye diseases, ocular trauma, patients who had already undergone ophthalmic surgery, alterations of ocular mobility, and binocular collaboration. As a result, 23 patients were excluded. Therefore, the study sample comprised a total of 100 patients. Among them, 25 were men (25%) and 75 were women (75%), with an average age of 32.63 years (standard deviation = 15.37; range, 10-68 years). The recruited sample was examined by the same calibrated personnel through orthoptic evaluation tests: visual acuity test, evaluation test of ocular alignment (cover test [CT], corneal bright reflex test, or Hirschberg test), ocular motility, convergence test, and Lang test [Figure 2].[2,25,26] A second clinical form, developed specifically for the study, was filled in with the orthoptic data [Figure 3].

# STATISTICAL ANALYSIS

A descriptive and statistical analysis was then carried out using the Statistical Package for the Social Sciences software (IBM: Armonk, New York, US). To verify the first objective, a z test was performed, significant when P < 0.05; to clarify the second objective, a frequency analysis of vision defects was conducted, significant when

the variable considered occurs in more than 50% of the sample; to clarify the third objective, an association between variables with a chi-square test and a difference between averages with a parametric Student's t-test were performed, both significant when P < 0.05; and to clarify the fourth objective, the scientific criteria of Bradford Hill were used.

### RESULTS

A large amount of data emerged from the analysis. Consequently, only the results that were most statistically significant and clinically interesting were reported. The results were listed according to the four objectives.

### **OBJECTIVE 1**

As regards vision problems, myopia occurred in 38% of the sample and 31% of the population; astigmatism in 40% of the sample and 24% of the population; hyperopia in 22% of the sample and 25% of the population; and strabismus in 4% of the sample and 3% of the population (P < 0.001) [Figure 4].

### **O**BJECTIVE **2**

The most frequent vision defect in the sample was oculomotor dysfunctions. In particular, of 100 patients, 92% had phoria, 3% had tropia, and 5% had orthophoria [Figure 5].

### **OBJECTIVE 3**

One of the most important associations was found between DDR and ocular convergence. Of 53 patients without DDR, 34 (64%) had normal convergence and 19 (36%) had decreased convergence, whereas of 47 patients with DDR, 19 (40%) had normal convergence and 28 (60%) had decreased convergence (P < 0.05) [Figure 6].

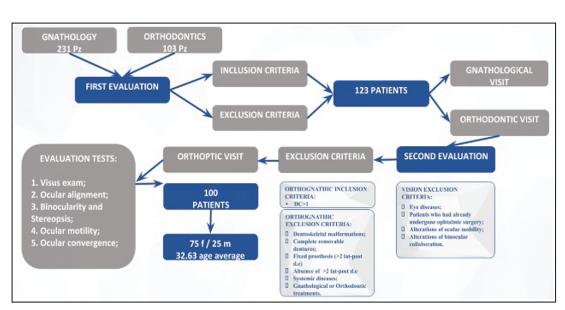
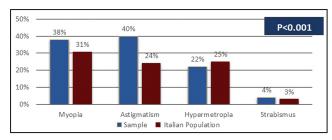


Figure 2: Gnathological medical record

ORTHOPTIC MEDICAL RECORD							
DATE							
SURNAME	NAME						
AGE	TELEPHONE NUMBER						
MEDICAL HISTORY							
AUTOREF RIGHT EYE	LEFT EYE						
AOTOREI RIGHTETE	ELITE II						
LAF in use RIG <u>HT EYE</u>	LEFT EYE						
VISUS (without correction)							
(with correction)							
CORNEAL BRIGHT REFLEX TEST							
Near ————————————————————————————————————	Far						
СТ							
OCIU AD MOTU ITV. Hymorfu natio							
Hypofunction	n						
Typoranetion							
CONVERGENCE TEST:							
LANG TEST:							
ABNORMAL HEAD POSITION:							

Figure 3: Orthoptic medical record



**Figure 4:** Comparison of frequencies of vision alterations between the sample and the Italian population

Another important association was found between asymmetry and astigmatism. Of 41 patients without asymmetry, astigmatism was absent in 19 (46%) and present in 22 (54%). Of 59 patients with asymmetry, astigmatism was absent in 41 (70%) and present in 18 (30%) (P < 0.05) [Table 1].

In addition, asymmetry was found statistically associated with oculomotor deviations. In particular, of 59 patients with asymmetry, 56 (95%) had phoria, 3 (5%) had tropia,

and 0 (0%) had orthophoria. Of 41 patients without asymmetry, 36 (88%) had phoria, 0 (0%) had tropia, and 5 (12%) had orthophoria (P < 0.01) [Figure 7].

Finally, a significant association was found between headache and emmetropia. Of 40 patients without headache, emmetropia was absent in 23 (58%) and present in 17 (42%), whereas of 60 patients with headache, emmetropia was absent in 47 (78%) and present in 13 (22%) (P < 0.05) [Table 2].

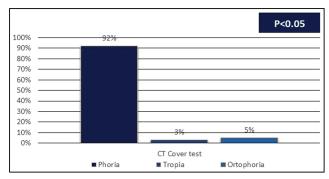
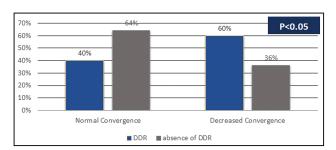


Figure 5: Prevalence of heterophorias in the sample



**Figure 6:** Association between disc displacement with reduction and convergence

Headache was also found significantly associated with hyperopia. Of 40 patients without headache, hyperopia was absent in 36 (90%) and present in 4 (10%). However, of 60 patients with headache, hyperopia was absent in 42 (70%) and present in 18 (30%) (P < 0.05) [Table 3]. The difference between the average values of gnathological algic symptoms and the ones of astigmatism, resulted statically significant.

The average values of arthralgia were equal to 1.9 when astigmatism was absent and equal to 2.1 when it was present. The average values of myalgia were equal to 1.36 when astigmatism was absent and equal to 2.1 when it was present. The average values of headache were equal to 1.93 when astigmatism was absent and equal to 2.65 when it was present. The average values of neck pain were equal to 2.17 when astigmatism was absent and equal to 2.33 when it was present (P < 0.05) [Table 4].

The results of associations between vision defects and the others DC/TMDs were not reported because of the low number of patients affected by. No associations between the subluxation and vision defects resulted statistically and/or clinically important to be shown.

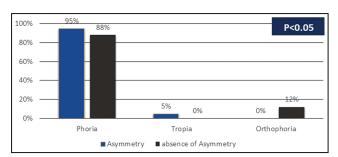


Figure 7: Association between asymmetry and oculomotor deviations

a. Contingency table			Asym	Total	
			Absence	Presence	
Astigmatism	Absence	Count	19	41	60
		Stand. residues	-1.2	1.0	
	Presence	Count	22	18	40
		Stand. residues	1.5	-1.2	
Total		Count	41	59	100
b. Chi-squared test	Value	df	Asymp. sig. (2 sides)	Exact sig. (2 sides)	Exact sig. (1 sides)
Pearson Chi-squared	5.938a	1	.015		
Continuity correction <sup>b</sup>	4.965	1	.026		
Likelihood ratio	5.940	1	.015		
Fisher exact test				.021	.013
Linear-linear association	5.878	1	.015		
Number of valid cases	100				

<sup>&</sup>lt;sup>a</sup>·0 cells (,0%) have an expected count of less than 5. The minimum expected count is 16,16

 $<sup>^{\</sup>text{b}}$ Calculated only for a table 2  $\times$  2

Table 2: Association	botwoon boadac	he and emmetronia	(a) Contingonov	table: (b) Cl	i covered test
Table 2: Association	- netween neadac	ne and emmetrobia.	. (a) Contingency	table: (b) Ci	II-squared test

a. Contingency table			Hea	Total	
			Absence	Presence	
Emmetropia	Absence	Count	23	47	70
		Stand. residues	9	.8	
	Presence	Count	17	13	30
		Stand. residues	1.4	-1.2	
Total		Count	40	60	100
b. Chi-squared test	Value	df	Asymp. sig. (2 sides)	Exact sig. (2 sides)	Exact sig. (1 side)
Pearson Chi-squared	4.728a	1	.030		
Continuity correction <sup>b</sup>	3.808	1	.051		
Likelihood ratio	4.681	1	.030		
Fisher exact test				.044	.026
Linear-linear association	4.680	1	.031		
Number of valid cases	100				

<sup>&</sup>lt;sup>a</sup>·0 cells (,0%) have an expected count of less than 5. The minimum expected count is 12,12

Table 3: Association between headache and hyperopia. (a) Contingency table; (b) Chi-squared test

a. Contingency table			Heada	Total	
			Absence	Presence	
Hyperopia	Absence	Count	36	42	78
		Stand. residues	.9	7	
	Presence	Count	4	18	22
		Stand. residues	-1.6	1.4	
Total		Count	40	60	100
b. Chi-squared test	Value	df	Asymp. sig. (2 sides)	Exact sig. (2 sides)	Exact sig. (1 side)
Pearson Chi-squared	5.801a	1	.016		
Continuity correction <sup>b</sup>	4.675	1	.031		
Likelihood ratio	6.292	1	.012		
Fisher exact test				.025	.013
Linear-linear association	5.742	1	.017		
Number of valid cases	100				

<sup>&</sup>lt;sup>a</sup> 0 cells (.0%) have an expected count of less than 5. The minimum expected count is 8,89

Among the nonsignificant associations, although clinically important and widely discussed in the literature, the association between dental class and refractive defects has to be highlighted.

Of 45 patients with Class I malocclusion, 16 (36%) had myopia, 17 (38%) emmetropia, 7 (16%) hyperopia, and 17 (40%) astigmatism. Of 44 patients with Class II malocclusion, 15 (34%) had myopia, 10 (23%) emmetropia, 14 (32%) hyperopia, and 17 (39%) astigmatism. Of 11 patients with Class III malocclusion, 8 (73%) had myopia, 3 (27%) emmetropia, 1 (9%) hyperopia, 6 (56%), and astigmatism (P > 0.05) [Figure 8].

### **OBJECTIVE 4**

The consistency and the coherence criteria were verified. The strength, the specificity, and the temporal sequence criteria were not verified.

# **DISCUSSION**

Compared to the reference values of Italian population, most of the frequency values of vision defects were found to be higher in the study sample: myopia increased from 31% to 38%; astigmatism increased from 24% to 40%; hyperopia decreased from 25% to 22%; and strabismus increased from 3% to 4%.[2,3] The incidence rates of the same defects were also confirmed in other studies.<sup>[27]</sup> Considering that frequencies of most of the vision defects increased in the study sample, a positive relationship between the two systems can be confirmed. The most frequent vision dysfunction evaluated with the CT was oculomotor deviation. The most frequent vision dysfunction evaluated with the CT was oculomotor deviation: in particular, phoria or latent strabismus (92%), tropia or manifest strabismus (3%). Therefore, only 5% of the whole sample had

<sup>&</sup>lt;sup>b</sup>.Calculated only for a table 2 × 2

 $<sup>^{\</sup>text{b}}$ .Calculated only for a table  $2 \times 2$ 

Table 4: Differences between the average values of gnathological algic symptoms and astigmatism. (a) Group statistics; (b) contingency table

a. Group statistics	Emmetropia	n	Average	Std. deviation	Std. error average
Arthralgia	Presence	30	2.07	1.484	.271
	Absence	69	1.94	1.740	.209
Myalgia	Presence	30	1.67	1.729	.316
	Absence	69	1.65	1.713	.206
Headache	Presence	30	1.67	1.709	.312
	Absence	69	2.46	1.668	.201
Neck pain	Presence	30	2.57	1.612	.294
	Absence	69	2.09	1.687	.203
b. Contingency table			t Test	for equality of averages	
			Sig. (2-tailed)	Difference between	Difference standard

b. Contingency tai	oie	t fest for equality of averages			
		Sig. (2-tailed)	Difference between	Difference standard	
			averages	error	
Arthralgia	Take equal variances	.733	.125	.365	
	Don't take equal variances	.717	.125	.342	
Myalgia	Take equal variances	.969	.014	.376	
	Don't take equal variances	.969	.014	.377	
Headache	Take equal variances	.032	797	.367	
	Don't take equal variances	.036	797	.371	
Neck pain	Take equal variances	.191	.480	.364	
	Don't take equal variances	.185	.480	.358	

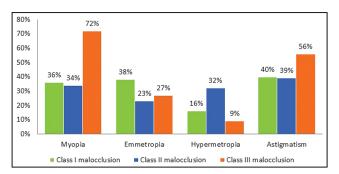


Figure 8: Association between dental class and refractive defects

orthophoria. A first very interesting significant association, as it adds a value to the hypothesis already present in the literature, is the association of the clicking and the DDR with ocular convergence. [22] In this study, the convergence deficit was found in 60% of patients with DDR. Also, in the reference study, the convergence deficit occurred more frequently in patients with DDR (22%) than in healthy controls (19%), but not significantly. Tissue inflammation and algic symptoms, due to progressive modification of joint structures, could affect the response at oculomotor level and could lead to hypersensitivity, induced by central sensitization, temporal sum, and activation of glial cells.[28] It can be postulated that the alteration of binocular motility may be due to a dysfunction at the level of the upper colliculus, center of visual, and somesthetic and proprioceptive afferences, involved in motor postural and gaze control.[23] One might think that the continuous intra-articular nociceptive

stimulus could reduce the activation mechanism of the motoneurons of the extrinsic eye muscle and thus alter the maintenance of proper eye convergence.

Mandibular asymmetry was also found to be significantly associated with astigmatism. frequencies of astigmatism were found to be lower in patients with asymmetry (30%) than in those without asymmetry (54%). The observed data did not coincide with the expected data. One could, in fact, think that an alteration at the osteobasal level could be associated with an asymmetry at the corneal level. But the astigmatism is an anatomical vision defect that it is difficult to be influenced by neurofunctional and muscular mechanisms. Another important association was found between asymmetry and oculomotor deviations evaluated with CT. The frequency of orthophoria was found to be higher in patients without asymmetry (12%) compared to those with asymmetry (0%). Therefore, it could be stated that in the presence of asymmetry, the risk of motor eye deviations increases.

One of the orthodontic and gnathological variables most frequently associated with vision defects was headache. It was observed that in patients with headache (59%), the presence of emmetropia was very low (22%) and the absence of it was very high (78%). Therefore, most of the patients with headache had a refractive defect, especially hyperopia. It occurred more frequently when headache was present (30%) as compared to when it was absent (10%). Another important correlation was found between average values of gnathological symptoms

and astigmatism. All average values of gnathological symptoms (arthralgia, myalgia, headache, and neck pain) were found to be increased when astigmatism was present as compared to when it was absent. In particular, the average value of headache increased from 1.93 to 2.65. Although from a gnathological point of view the headache could create a visual dysfunction, from an orthoptic point of view the headache is a consequence of vision dysfunctions. In fact, it is a very frequent symptom in vision defects due to the physical effort of the subject to improve vision. [29] It is also true that headache has been diagnosed by anamnesis as a pain symptom present at least twice a week but has not been evaluated by the neurologist. This limit prevents the nosological classification of the type of headache and the definition of the influence between it and the oculomotor alterations.

In the literature, several studies showed a correlation between vision defects and malocclusion. In this study, none of these associations were significant. In the largest study, taken in consideration as a reference, a higher frequency of hyperopia (59%) and astigmatism (50%) was found in Class I malocclusions, and a higher frequency of myopia (50%) in Class II malocclusions. In this study, hyperopia was found to be more frequent in Class II malocclusions (32%), whereas astigmatism (56%) and myopia (72%) were found to be higher in class III malocclusions. [16] The difference between the two studies lies in the size of the sample, which is 1326 in the reference study and 100 in this study, and in the type of malocclusion considered, skeletal in the first study and dental in the second one. The results obtained in the study may be due to the consideration of the dental class. It was not possible to define the type of relationship between TMDs and vision diseases. The consistency criterion was satisfied; the strength one could not be met because this study only analyzed a group of subjects exposed to the hypothetical risk (dysfunctional patients) and not a group of subjects not exposed (healthy). However, the study sample was compared with the Italian population, and it was found that the study sample was out of norm. The specificity criterion is not met if we consider each TMD (DC/TMD), whereas it can be defined positively if we consider temporomandibular disturbances as anomalies capable of disturbing biological functions. The temporality criterion cannot be applied because the time of the onset of the temporomandibular disorder with respect to the vision defect has not been detected. The criterion of coherence seems to be satisfied as the association is biologically plausible, given the numerous anatomical, neurological, and functional biological correlations between the stomatognathic apparatus and the oculomotor system.

### **CONCLUSION**

A statistically and clinically significant relationship between some orthoptic and gnathological variables seemed to exist. Frequencies of many vision defects, such as oculomotor dysfunctions and most of refractive defects, seemed to be high in patients with TMDs. The most interesting associations were found between functional or skeletal orthodontics and gnathological alterations and oculomotor disorders. The type of relationship or the direction of influence between the two entities could not be established. Further studies by enlarging the sample should be conducted in the future to define the relationship. Patients with these types of vision defects should be included in a diagnostic multidisciplinary protocol. Although there is a positive significant association between the two anomalies, no orthodontic treatment is currently justified to correct an oculomotor alteration and vice versa.

### **A**CKNOWLEDGEMENT

Nil.

### FINANCIAL SUPPORT AND SPONSORSHIP

Nil.

# **C**ONFLICTS OF INTEREST

There are no conflicts of interest.

### **A**UTHORS CONTRIBUTIONS

C.V., E.S.; performed experiments, analysed data and co-wrote the paper. S. P., A.S.; contributed to sample preparation. L. G.; contributed to the interpretation of the results. C. D. P., G.G.; devised the project, the main conceptual ideas and proof outline. Each group of authors analyzed the results, according to their specialty, all participated in the discussion and actively contributed to the drafting, CDP carried out the overall and definitive revision of the manuscript.

### ETHICAL POLICY AND INSTITUTIONAL REVIEW BOARD STATEMENT

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee (Approval no. 12/2018 – 0000106) and with the Helsinki declaration (1964) and its later amendments or comparable ethical standards.

### PATIENT DECLARATION OF CONSENT

Informed consent was obtained from all individual participants included in the study.

### **D**ATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, [C.V.], upon reasonable request.

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