Heavy metals and pain in the dysfunctional patient

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Summary

***Aims*. The aim of this research is to verify the quality and quantity of heavy metals (HM) of dental origin in TMD patients. *Methods*. A population of 100 subject was studied and divided in two homogeneous groups: Study Group (SG) and Control Group (CG). Organism heavy metals were tested by a spot sampling method in which the first urine of the day, through Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), were analyzed. The results obtained were compared with reference values (RV) of Italian people. Descriptive statistical analysis and student's t-test has been applied (statistical significance for p > 0.05). *Results*. The SG presented the absolute highest levels of HM compared to the CG (p=0.787). As regards the relation between pain and HM, the subjects that refer "severe/very severe" values of pain present the highest levels of HM in urines. *Conclusions*. The obtained results seem to highlight a possible direct proportionality between the level of pain the increase of the concentration of heavy metals in all the examined groups and subgroups.**

Key words: heavy metals, pain, TMD.

Introduction

Temporomandibular Disorders (TMD) are a group of pathologies determined by an interaction of structural, psycho-socio-environmental and genetic elements (1,2). In the past some authors (3,4) developed the idea that inflammation might be a pivotal factor in the pathogenesis of such disorders. Other authors (5,6) claim that the production of free radicals, pro-inflammatory and others neuropeptides, may lead to localized inflammation, chronic tissue modifications, and to the development of neurogenic sensitization.
Toxins, free radicals and metals can caused inflammation phenomena and cellular homeostasis modifications, generating pathologies and sometimes even irreversible damages (7-13). Among these, heavy metals are particularly relevant. They can penetrate the organism through numerous ways (food, medicines, cosmetics), including materials routinely used in dentistry, leading to a deficiency in some vital functions (14-18).
An elevated concentration and accumulation of free radicals in biological tissues may cause oxidative stress (19-24). Among heavy metals, Mercury (Hg), deriving from alimentary, iatrogenic, cosmetic factors and mostly from amalgam dental fillings, can cause a particularly relevant chronic intoxication (17,25).
The aim of this research is to quantify the quality and quantity of heavy metals of dental origin in TMD patients. This comparison is based on the possible relationship between these pathological conditions and the inflammatory processes. This research project requires the comparison between three groups of randomly selected subjects: a group of dysfunctional patients with metal manufacts in the oral cavity, a group of dysfunctional patients without any metals and a group of non-dysfunctional subjects, without any metals in the oral cavity.

Materials and methods

A consecutive series of patients has been selected, all spontaneously joining the Service of Clinical Gnathology of the Department of Odontostomatological and Maxillofacial Sciences of the University of Rome "Sapienza", from February 1st to November 30th 2011, for a total of 200 patients. These were divided into two groups: a *Study Group* (SG) and a *Control Group* (CG), according to the correspondence to the following criteria:
Inclusion criteria for the Study Group: 1. Presence of TMD belonging to groups I, II, III of the Axis I, according to the RDC/TMD classification, or problems of articular dysfunction related to all phases of condylodiscal incoordination, from mandibular dislocation to arthrosis, 2. Presence of one or more conservative or prosthetic metal manufacts, minimum 2 amalgam fillings and/or 1 fixed-removable prosthesis.

Inclusion criteria for the Control Groups: The study provides the composition of two control groups, 1 and 2. The first (CG1) is made up of TMD patients with the above mentioned characteristics who do not present any conservative or prosthetic metal manufacts inside the oral cavity. The second (CG2) is made up of healthy subjects without any conservative or prosthetic metal manufacts inside the oral cavity.
Exclusion criteria for all the groups: 1. Positivity to the Axis II of the classification RDC/TMD 2. Occurrence of well-known local and/or systemic diseases that may alter the inflammatory condition of the examined subjects. 3. Presence of other metals in the body. 4. Subjects who do not accept to be part of the survey.
Suitable patients for the research are in the number of 50 for the Study Group and 15 for the Control Group. The scarce number of dysfunctions in this last group indicates the difficulty in finding patients without any prosthetic or conservative reconstructions among the subjects affected by TMD. Thus, in order to have an equal number of participants in the two groups, 35 subjects have been recruited in the Control Group among the medical and the nursing staff of the Service and students from the degree course in "Dental Sciences" at the University of Rome "Sapienza".
The total patient population is thus made up of 100 subjects, of an average age of 41, mainly from the female sex, 50 belonging to the Study Group (SG) and 50 to the Control Group (CG). (Tab. 1-2)
The SG is made up of TMD-affected patients, whose level of heavy metals has been evaluated and compared with the intensity and typology of the dysfunctional pathology; the CG, 50 subjects without any metals in the oral cavity, is made up of 15 dysfunctional patients (CG1) more 35 non-dysfunctional subjects (CG2). The CG1 has been compared with the SG to evaluate the relationship between heavy metals, intensity and dysfunctional typology.
All the subjects involved have been informed in advance about the study, its aims and the potential risks, and have been given an informed consent paper. All have undergone a health checkup, consisting of a general medical examination performed by a specialist (an anesthetist), and requiring basic hemato-chemical examinations in order to highlight the absence of any in-progress inflammation. The study has been carried out randomly, using a spot sampling according to Araki S.' method, and providing patients with a sterile 50cc container to collect early morning urines, which has been then returned to the Service of Clinical Gnathology.
Urines analysis: urines have been analyzed by the Laboratory of Geochemistry of the Department of Earth Sciences of the University of Rome "Sapienza". Analytical data have been elaborated in cooperation with the Department of Chemistry and Medicine Technologies of the University of Rome "Sapienza".
The *assay* of the heavy metals present in dental alloys, named "ODmetals" (Tab. 3), has been performed, as well as of other toxic metals, for a total number of 22 metals examined (Tab. 4), also to test the subjects' degree of intoxication.
A *measurement of the relative density* has been carried out through a refractometer, in order to correct the results according to Araki S'method (26). The samples have then been analyzed through the *ICP-MS mass spectrometry* (Inductively Coupled Plasma-Mass Spectrometry). Data interpretation is possible by confrontating the results with the reference values (RV) for Italy (Tab. 5), elaborated by the National Institute for Health (ISS) of Rome, that measures the presence of metals in the Italian population between 1990 and 2009 (27).
These values have been compared with those resulting from the analysis of the collected samples, in order to check if they were normal. The research on the possible correlation between the presence of heavy metals

Table 1. Distribution of age in SG e CG.

AGE	SG	CG
Minimum	23	22
Maximum	75	62
Mean	47,92	28,59
Median	51,5	27,5

Table 2. Distribution of gender in SG e CG.

GENDER	SG	CG
Female	39	31
Male	11	19

Table 3. OD METALS.

Heavy metals of dental origin
Manganese (Mn)
Copper (Cu)
Zinc (Zn)
Molybdenum (Mo)
Cobalt (Co)
Tin (Sn)
Nickell (Ni)
Mercury (Hg)
Antimony (Sb)
Indium (In)
Titanium (Ti)

Table 4. Heavy metals examined.

• Aluminum (Al)	• Colbalt (Co)
• Iron (Fe)	• Cadmium (Cd)
• Manganese (Mn)	• Tin (Sn)
• Copper (Cu)	• Nickel (Ni)
• Arsenic (As)	• Lead (Pb)
• Cesium (Cs)	• Mercury (Hg)
• Lithium (Li)	• Antimony (Sb)
• Rubidium (Rb)	• Indium (In)
• Zinc (Zn)	• Titanium (Ti)
• Strontium (Sr)	• Barium (Ba)
• Molybdenum (Mo)	• Uranium (U)

Table 5. Concentration of metals in the urine for the Italian population from 1990 to 2009.

Metal	***Reference value (RV)***	***Other studies***
	Urine (μg/L)	*Average ± ds Urine (μg/L)*
Al	2,3-19,5 (1990)	10,9±1,06 (AAS, ICP-AES 1990) 5,36±3,76 (ICP-MS 2009)
As	2,3-31,1 (1990)	16,7±1,9 (AAS, ICP-AES, NAA 1990)
Cd	0,38-1,34 (1990)	0,86±0,06 (AAS, ICP-AES 1990 0,81±0,53 (ICP-MS 2009)
Co	0,18-0,96 (1990)	0,57±0,1 (AAS, ICP-AES, NAA 1990) 0,24±0,18 (ICP-MS 2009)
Cs	0,1-17,5 (1990) 2,00-6,82 (2005)	8,1±1,5 (NAA 1990) 4,52±2,24 (ICP-MS 2005) 12,9±8,3 (ICP-MS 2009)
Cu	4,20-50 (1990)	23±6,9 (AAS, ICP-AES, NAA 1990) 12,9±7,0 (ICP-MS 2009)
Fe		8,70±6,27 (ICP-MS 2009)
Hg	0,1-6,9 (1990)	3,5±0,2 (AAS, ICP-AES, NAA 1990) 1,92±1,60 (ICP-MS 2009)
Li		17,3±13,6 (ICP-MS 2009)
Mn	0,12-1,90 (1990)	1,02±0,05 (AAS, ICP-AES 1990) 0,22±0,10 (ICP-MS 2009)
Mo		36,9±16,9 (ICP-MS 2009)
Ni	0,06-1,74 (1990)	0,9±0,11 AAS, (ICP-AES 1990) 0,87±0,50 (ICP-MS 2009)
Pb	12,0-27,0 (1990)	17 ± 0,46 (AAS, ICP-AES 1990) 1,80 ± 1,40 (ICP-MS 2009)
Rb	284-4.096 (1990)	2.190 ±203 (NAA 1990)
Sn		0,90±0,64 (ICP-MS 2009)
Sr		154±91 (ICP-MS 2009)
Zn	266-846 (1990)	456±58 (AAS, ICP-AES, NAA 1990) 356±236 (ICP-MS 2009)
Ba	0,67-3,68 (2005)	2,7±0,5 (AAS, ICP-AES, NAA 1990) 1,77±1,30 (ICP-MS 2005) 1,24±0,78 (ICP-MS 2009)
In		< 0,15 (NAA 1990)
Sb	0,19-1,10 (1990) 0,02-0,12 (2005)	0,79±0,07 (AAS, ICP-AES, NAA 1990) 0,07±0,04 (ICP-MS 2005)
Ti	0,07-0,7 (1990) 0,02-0,17 (1994)	0,42±0,09 (AAS 1990) 0,07±0,03 (ICP-MS 1994)
U		< 0,1 NAA (1990)

over the threshold levels and the algic symptomatology in dysfunctional patients, has been focused mainly on the Study Group, where the totality of the patients is affected by TMD. Subsequently, the results have been confronted with the symptomatology of the CG1 patients, representing 30% of the whole sample.

The following pathological and symptomatic aspects have been considered:

I. ***Articular problems:*** according to the RDC/TMD classification, the pathologies I, IIa, IIb, and IIIc (Axis I) have been taken into account (1). These have been considered either *mild* or *severe* according to the presence or absence of pain, classified as below, and to the level of chronicity, through the relation between *onset time/clinical observation time*.

II. ***Main symptoms:*** TMJ pain (IIIa), and MUSCULAR pain (MM).

Pain has been measured with the subjective Verbal Numeric Scale (VNS), according to an index of seriousness subdivided in four categories: mild (<30); moderate (30-50); severe (50-70); very severe (>70).

III. ***Comorbidity***: among the possible co-morbidities with TMD, the most frequently considered are headache and cervicalgia, anamnestically described by patients, measured with the VNS scale and subdivided with reference to the above described index of seriousness.

Heavy Metals analysis*:* evaluates the quantity and the typology of those metals presenting higher values than the RV for the Italian population, paying particu-

lar attention to the most frequent heavy metals in dental preparations, especially Mercury (Hg).

Dysfunctional problem analysis: correlates the dysfunctional characteristics, such as pathology and pain, with the occurrence of heavy metals in urines, in order to highlight a possible relation between the two. As regards the SG, in order to carry out a more significant check of the relation between the quantity of heavy metals and the seriousness of the symptoms, subjects with a higher total level of heavy metals (Gr1) and metals present in dental preparations (Gr1OD) have been compared with patients presenting a minor level of total heavy metals (Gr3) and of the most frequent metals in dental preparations (Gr3OD) (Tab.6, 7).

To verify the reliability, the data have been crossed for metals and pathology/symptomatology, the various metals have been quantified in the selected groups, and subsequently, the presence of metals in the different dysfunctional typologies has been also evaluated (Fig. 7-16 in the results paragraph).

Statistical analysis: the data obtained from urine analysis and those related to the pathological and symptomatological aspects are not time-variant, that is, we do not have any data referring to any 0 time and subsequent phases; thus, a *descriptive statistic analysis* has been carried out, in order to synthesize the available data through graphic instruments (histograms), as well as indexes (the mean of variances from RV) describing the most important aspects. In particular, this kind of analysis has been of both qualitative (presence/absence of heavy metals in urines and of a dysfunctional symptomatology) and quantitative (level of heavy metals) nature. For the quantitative data, "Student's t-test" has been applied (*the difference between the observed means is not significant for p<0,05*).

Results

A large quantity of data have emerged from the analysis and the comparisons between the various sample groups, consequently only the most significant for the aims of this research have been reported. Data have first been observed considering the presence of heavy metals, and subsequently analyzed in the different sample groups.

Table 6. SG subdivision according to the number of total metals.

	Pt. N°	% of SG	Total heavy metals (19)
Gr1	6	12 %	> 10metals
Gr2	26	52%	5< metals ≤ 10
Gr3	18	36%	1≤ metals ≤ 5

Table 7. SG subdivision according to the number of OD metals.

	Pt. N°	% of SG	OD Metals (10)
Gr1OD	4	8%	> 6 ODmetals
Gr2OD	14	28%	3< ODmetals ≤ 6
Gr3OD	32	64%	1≤ ODmetals ≤ 3

Heavy metals analysis

The whole studied population presents, for most of the metals which are taken into account, urine values superior to the "*reference values for Italian population (RV)*" (27). Each subject, independently from the group, presents levels which are over the threshold for at least 4 of the 19 metals taken into account. (Figs. 1-2)

Yet, the Study Group presents the absolute highest levels, compared to the whole Control Group. The higher presence of metals in SG than in CG, in terms of variance *entity* from RV, is well highlighted from the results obtained by the **Student's t-test**, which made the comparison possible among the *variance means from RV (vmRV)* of all metals:

Total metals (19): t = 0.2715;
P (level of significancy) = 0.7876

Another interesting result, in terms of variance entity from RV, is obtained by the internal CG group comparison, that is, between the CG1 and CG2 subgroups. The analysis of the *vmRV,* for all the examined metals and considering these two groups separately, has highlighted that CG1 presents a larger number of heavy metals than CG2.

Student's t-test has actually proved significant:

Comparison GC1 – GC2: t = 0,1193;
P (level of significancy) = 0,9057

Another datum that has emerged analyzing metals in the different samples, concerns the so-called "OD metals" group (10 metals), namely those heavy metals that can be found in dental alloys and in amalgams, which are thus of specific interest for us.

Six of these present a higher variance from the RV in the SG patients (Tab. 8), while the remaining 4, with levels over the threshold, are more frequent in the CG patients (Tab. 9).

The most frequently occurring elements in SG patients, particularly Mercury (Hg), are by far the most represented metals in dental materials. This datum confirms and reinforce the sample division that has been made in the research.

The **Student's t-test** is also significant:

OD Metals (10): t = 0.9735;
P (level of significancy) = 0.3448

The results emerging from the statistic analysis and the descriptive analysis, which is expressed in percentages, confirm each other, thus resulting significant.

"OD metals" are present with higher values than RV also in patients who have prosthesis and/or orthodontic appliances (16 patients: 32% of the SG). In these patients the most occurring elements are Molybdenum (Mo) in 50% of the sample (8 patients), Iron (Fe) and Mercury (Hg) in 62% (10 patients), Antimony (Sb) in 69% (11 patients), and Titanium (Ti) in 100% of the sample (16 patients) (Fig. 3).

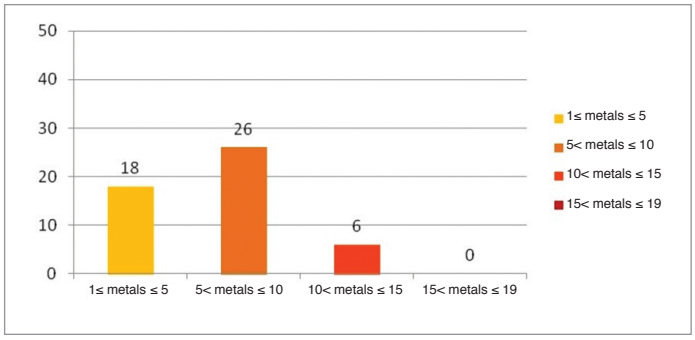

Figure 1. Distribution of metals increased in SG (50 patients).

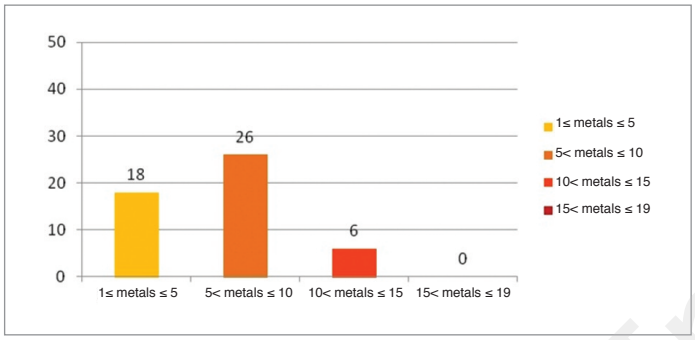

Figure 2. Distribution of metals increased in CG (50 patients).

Table 8. OD Metals more in SG.

Metal	***SG***		*CG*	
	N° Pt	**%**	N° Pt	%
Zinc (Zn)	12	**24**	5	10
Molybdenum (Mo)	19	**38**	8	16
Mercury (Hg)	32	**64**	9	18
Titanium (Ti)	30	**60**	11	22
Antimony (Sb)	38	**76**	17	34
Tin (Sn)	5	**10**	2	4

Table 9. OD Metals more in CG.

Metal	*SG*		***CG***	
	N° Pt	%	N° Pt	**%**
Cobalt (Co)	7	14	31	**62**
Nickel (Ni)	12	24	35	**70**
Indium (In)	0	0	3	**6**
Manganese (Mn)	12	24	35	**70**

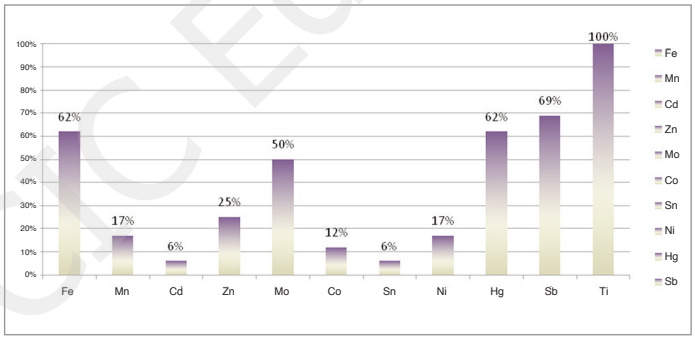

Figure 3. Metals with significant percentage deviancy in prosthetic/orthodontic patients.

The most interesting result with Mercury (Hg) is that the variance from the reference value is proportional to the number of amalgam fillings in the single patient's oral cavity (Fig.4); therefore, it seems that there might be a direct relation between the number of fillings and the increased release of metal ions in the oral cavity.

Dysfunctional problem analysis

The presence of heavy metals in the different dysfunctional pathologies has been evaluated, and in order to proof the results, the data have been crossed contrariwise; that is, in the different dysfunctional/symptomatological groups, metals have been quantified. To have a

complete vision and a more specific comparison of the data, the different groups have been compared: dysfunctional subjects with metal preparations in the oral cavity (SG: 50 patients, 100% of SG), the dysfunctional ones without any metal preparations in the oral cavity (CG1: 15 patients, 30% of the CG) and also those non-dysfunctional subjects without any metal preparations in the oral cavity (CG2: 35 patients, 70% di CG).

Articular problems: the data related to the pathologies belonging to the dysfunctional groups IIa (23 patients: 23% of the sample) and IIIc severe (7 patients: 7% of the sample) have resulted significant. Crossing them with the quantitative subdivision of the metals, it has resulted that in both pathologies the most frequent groups are Gr1 and Gr1OD.

As regards the the *IIa severe*, in a total of 13 patients Gr1occurs in 83% (19 patients) and Gr1OD in 100% (23 patients); Gr3 is present in 33% (7,59 patients), Gr3OD in 31% (7,13 patients), CG1in 27% (6,21 patients) and CG2 in 0% (0 patients). As regards the *IIIc severe*, in a total of 7 patients Gr1 present in 33% (2,31 patients), Gr1OD in 50% (3,50 patients), Gr3 in 17% (1,19 patients), Gr3OD in 12% (0,84 patients), and CG1 and CG2 in 0% (Figs.5-6).

Main algic symptomatology: TMJ pain (IIIa) and MM pain.

TMJ pain is mainly referred to as *severe/very severe*: in a total of 31 patients (48% of the sample) Gr1 occurs in 84% (26 patients), Gr1OD in 75% (23 patients), Gr3 in 55% (17 patients), Gr3OD in 50% (15

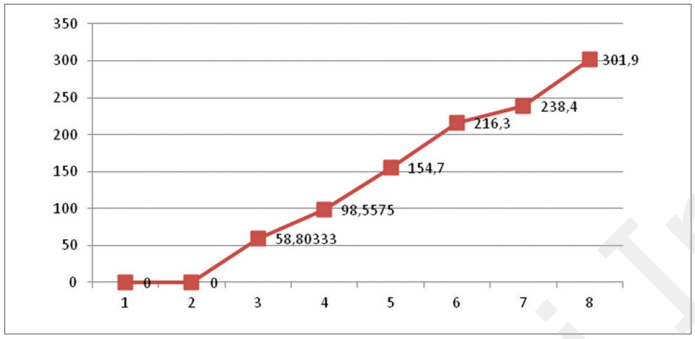

Figure 4. Hg amount in SG patients with amalgam fillings.

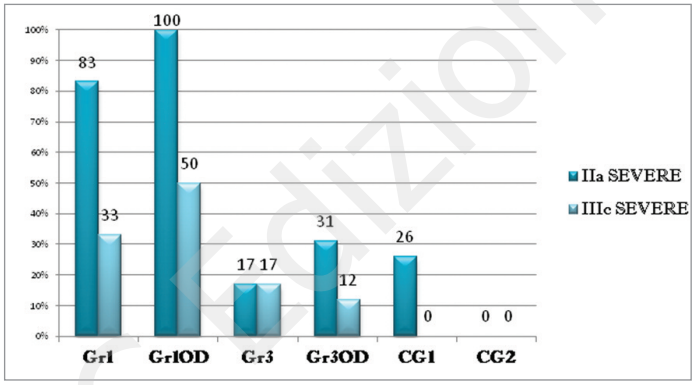

Figure 5. Metals – Articular problems.

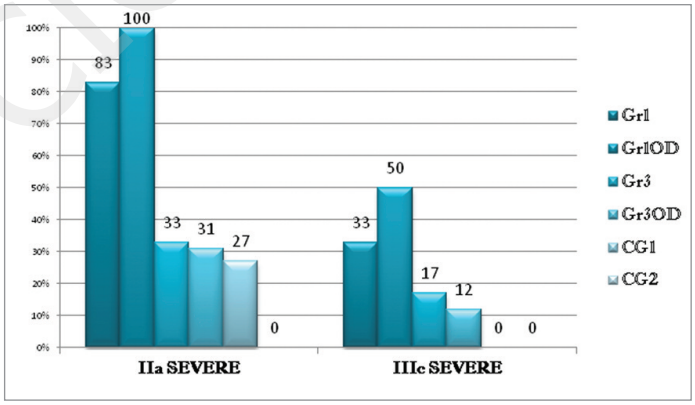

Figure 6. Articular problems – Metals.

patients), CG1 in 20% (6 patients) and CG2 in 0% (0 patients).
In all the examined groups TMJ pain has been defined *mild/moderate* by a minor percentage: in a total of 17 patients (26% of the sample) Gr1 is present in 17% (3 patients), Gr1OD in 25% (4 patients), Gr3 in 16% (3 patients), GrOD in 25% (4 patients), CG1 in 33% (6 patients) and CG2 in 0% (0 patients) (Figs.7-8).
Muscular pain has been mainly referred to as *severe*: in a total of 27 patients (41% of the sample) Gr1 Is present in 83% (22 patients), Gr1OD in 75% (20 patients), Gr3 in 44% (12 patients), Gr3OD in 34% (9 patients), CG1 in 27% (7 patients), CG2 in 0% (0 patients).
Muscular pain has been defined as *mild* by a lower percentage in all the examined groups: in a total of 8 patients (12% of the sample) Gr1 and Gr1OD are present in 0% (0 patients), Gr3 in 8% (0,64 patients), Gr3OD in 19% (1,52 patients), CG1 in 7% (0,56 patients) and CG2 in 0% (0 patients) (Figs. 9-10).
Comorbidity: headache and cervicalgia.
Headache is represented mainly as *severe/very severe* in all the examined groups: in a total of 36 patients (55% of the sample) Gr1 is present in 67% (24

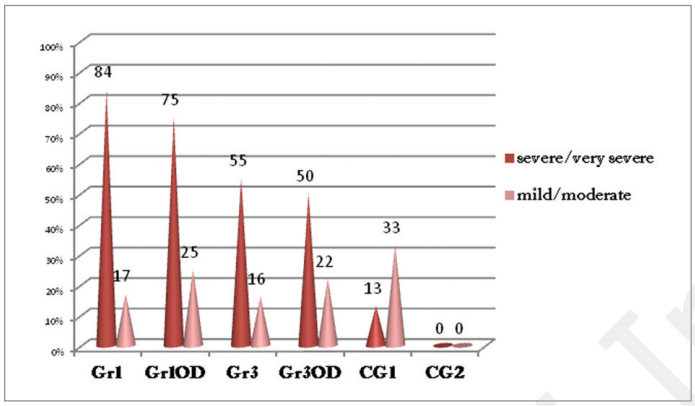

Figure 7. Metals – TMJ pain.

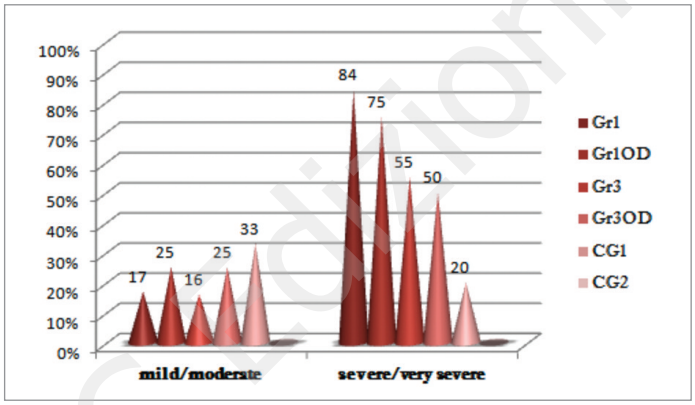

Figure 8. TMJ pain – Metals.

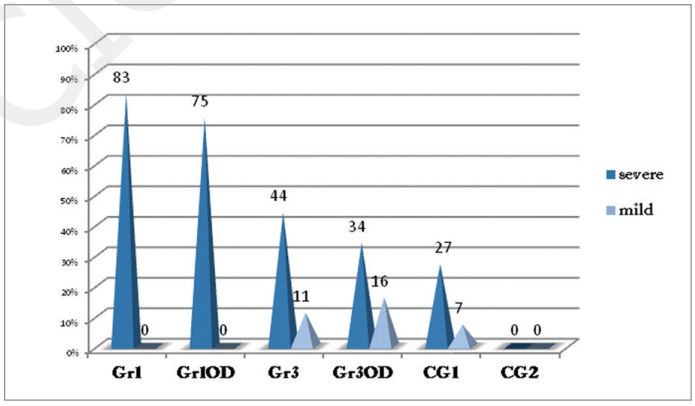

Figure 9. Metals - MM pain.

patients), Gr1OD in 75% (27 patients), Gr3 in 66% (24 patients), Gr3OD in 69% (25 patients), CG1 in 33% (12 patients) and CG2 in 0% (0 patients).
Headache has been defined as *mild/moderate*, instead, by a lower percentage of patients: in a total of 12 patients (18% of the sample) Gr1 and Gr1OD are present in 0% (0 patients), Gr3 in 1% (0,12), Gr3OD in 6% (0,72), CG1 in 54% (6,48 patients) and CG2 in 0% (Fig. 11, 12).
As regards *Cervicalgia*, this has also been defined as *severe/very severe*: in a total of 32 patients (49% of the sample) Gr1 and Gr1OD are present in 50% (16 patients), Gr3 in 67% (21 patients), Gr3OD in 63% (20 patients), CG1 in 26% (8 patients) and CG2 in 0% (0 patients). Cervicalgia has been defined instead as *mild/moderate* by a lower percentage of patients: in a total of 19 patients (29% patients), Gr1 is present in 33% (6,27 patients), Gr1OD in 25% (4,75 patients), Gr3 in 17% (3,23 patients), Gr3OD in 15% (2, 85 patients), CG1 in 47% (8,92 patients) and CG2 in 0% (0 patients) (Fig. 13, 14).

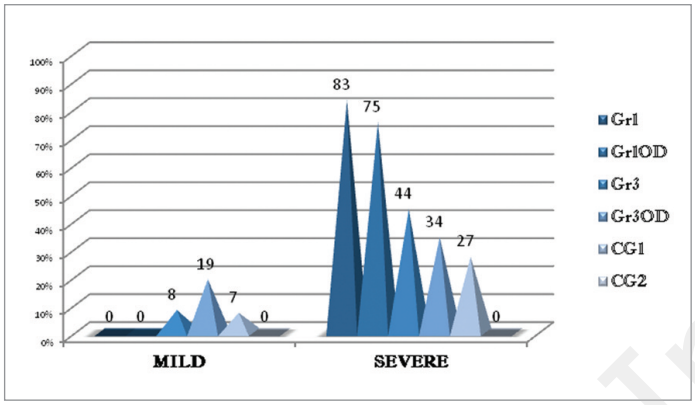

Figure 10. MM pain – Metals.

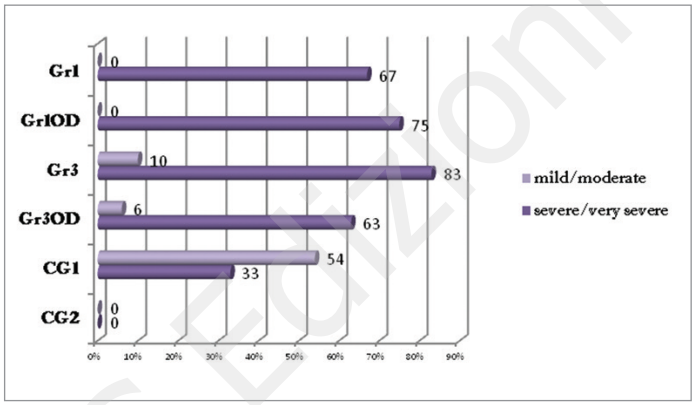

Figure 11. Metals – Headache.

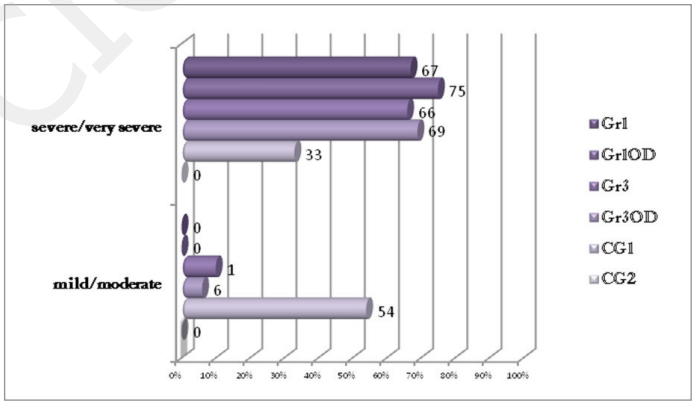

Figure 12. Headache – Metals.

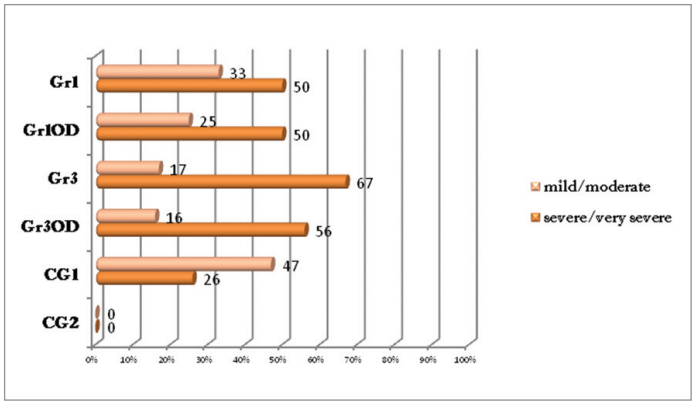

Figure 13. Metals – Cervicalgia.

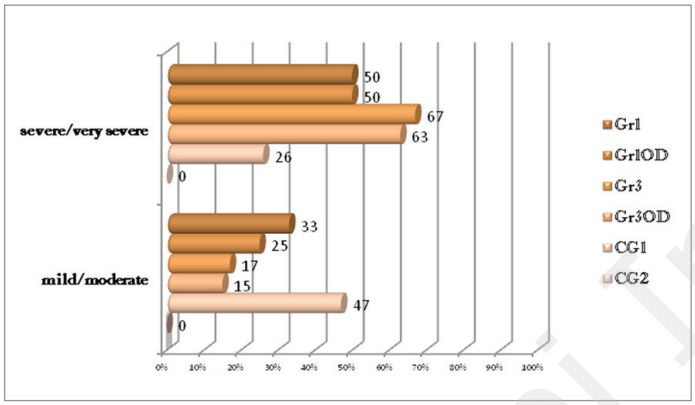

Figure 14. Cervicalgia – Metals.

Discussion

The above reported results agree with those studies that underline how much environmental exposition factors such as air, water, and food are relevant sources of contamination for the whole population, both healthy and affected by pathology (28,29). In fact, the CG1(dysfunctional subjects without dental preparations in the oral cavity) presents high concentrations of metals that may make the dysfunction worse. Even if within the limits of this sample, this is a presumable result because there is a directly proportional tendency between the concentration of metal ions and the exacerbation of the clinical symptomatology.

The obtained results, moreover, seem to coincide with those researches that show how dental materials, especially those present in the amalgams, can release metal ions in the organism (30) (Figs. 3-4).

As regards the considered **pathologies**, the most significant data are to be found in the **severe forms** (for the presence of pain), and in particular in the *Reducible disc displacement* (IIa according to the RDC/TMD), and the *TMJ arthrosis* (IIIc according to the RDC/ TMD). Insignificant, and thus not reported, are the dysfunctional forms related to the group of the *Irreducible disc displacement* (IIb according to the RDC/TMD; 3 patients: 6 % in SG-1 patient: 2% in CG). The scarce validity of the data for this groups is due to the scarcity of the sample, rather than to typology of dysfunction.

The **SG patients** with the more serious forms (IIa severe and IIIc severe) present a higher percentage of total heavy metals (Gr1, 6 patients: 12% of the SG), and metals from dental preparations (Gr1OD, 4 patients: 8% di SG). The percentage deviancy of this above mentioned serious sample is significant if confronted with the deviancy of patients presenting a lower level of heavy metals, both total (Gr3, 18 patients: 36% of SG) and OD metals (Gr3OD, 32 patients: 64% of SG).

Inside the **control group (CG1+CG2)** the *IIa severe* represents the 26% (4 patients) of the CG1 (15 patients: 30% of the CG) and *IIIc* the 0% (no patients), while in the CG2 there is no pathology represented (Figs. 5-6).

As regards the **main algic symptomatology** (TMJ pain, MM pain) in patients belonging to the **SG,** it is mainly **severe** or **very severe**, even if with different percentages according to the groups (84-83% of Gr1; 75% of Gr1OD; 55-44% of Gr3; 50-34% of Gr3OD). In CG the *TMJ pain* is defined mainly as **mild** (33% of CG), and *MM pain*, conversely, as **severe** (27% of CG). The most interesting evidence is that, in the Gr1and Gr1OD groups, we find the highest percentages of TMJ and MM pain referred to as *severe/very severe* (Figs. 7-10).

As regards **comorbidity** (headache and cervicalgia), it also proved **severe** or **very severe** in the **SG**, al-

though no pivotal percentage variances are found in the various subgroups (67-50% of Gr1; 75-50% of Gr1OD; 66-67% of Gr3; 69-63% of Gr3OD). The patients of the **CG**, on the contrary, report *lower values* (mild for 54-47% of the sample) (Figs. 11-14).

Conclusions

The obtained results seem to highlight a certain direct proportionality between dysfunction and algic symptomatology seriousness and the increased concentration of heavy metals in all the examined groups and subgroups.
This data may be linked to some hypotheses found in the literature. Among these, we remember those by De Bont and Stegenga (1997) (3,4) or Kacena at al. (2001) (31) on the relation between *inflammation* and *internal derangement* and tissue degeneration phenomena characterizing TMD, or Molina et al. (2011) (6), who link it to the appearance of articular and muscular pain. Milam et al. (1998) (32) and De Laat (2001) (5) find a correlation between the inflammation and the insurgence of an *oxidative stress* condition, that may further amplify both the tissue inflammatory reaction and the sensitization phenomena at the level of peripheral nervous system (33-36).
In particular, this research has pointed out, for the first time, how the accumulation of heavy metals released by dental preparations may be one of the factors of the inflammation and/or of its progression. Some metals can also cause an *oxidative stress* increase, being able to catalyze the decomposition of the hydrogen peroxide with the formation of oxydrilic free radicals, promoting inflammatory and degenerative processes as well as the appearance of muscular and articular algic symptomatologies (32,37).
The most relevant etiological hypothesis indicate that at the basis of dysfunctional pathologies there is a co-occurrence of different factors, without measuring their effective role (1,38,39). This research points out the necessity that both clinicians and researchers start taking into account the possible correlations between TMD and heavy metals in the oral cavity, also considering the frequent occurrence of both cases in the population. In any case, it is necessary to confirm these results by developing wider and deeper studies.

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