Rhinology

Maxillary fungus ball: zinc-oxide endodontic materials as a risk factor

Il Fungus Ball Mascellare: il materiale endodontico a base di ossido di zinco come fattore di rischio

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SUMMARY

The objective of this study was to demonstrate the correlation between endodontic treatment on maxillary teeth and fungus ball with inductively coupled plasma mass spectrometry measurement of zinc and other metals (barium, lead and copper) in fungus ball samples. Samples of normal maxillary mucosa were used as comparison. Metal concentration was also measured in several endodontic materials. A significant difference was found between the concentration of zinc and copper in fungus ball compared to normal mucosa. Metal distribution was more similar in fungus ball and in the endodontic materials tested than normal mucosa. The similar metal concentration in the endodontic materials and fungus ball suggests that endodontic materials play a role in the pathogenesis of fungus ball. Endodontic materials accidentally pushed into the maxillary sinus during endodontic treatments may play a crucial role. Dentists should be as careful as possible when treating maxillary teeth to avoid perforating the maxillary sinus floor; the use of zinc-free endodontic materials, as zinc is a metal that plays a pivotal role in fungus growth, should be encouraged.

KEY WORDS: Maxillary fungus ball • Zinc-oxide materials • Endodontic treatment • Inductively coupled plasma mass spectrometry

RIASSUNTO

L'obiettivo di questo studio è stato dimostrare la correlazione tra il trattamento endodontico su denti mascellari antrali e il Fungus Ball mascellare. mediante L'utilizzo della ICP-MS (Inductively Coupled Plasma Mass Spectrometry) è stata utilizzata per la misurazione della concentrazione di zinco e altri metalli (bario, piombo e rame) in campioni di Fungus Ball. Per il confronto sono stati utilizzati campioni di mucosa sana del seno mascellare. La concentrazione di questi degli stessi metalli è stata misurata anche in diversi materiali utilizzati per le terapie endodontiche. Vi era è una differenza significativa tra la concentrazione di zinco e rame nei campioni di Fungs Ball rispetto alla mucosa sana. La concentrazione del dei metallo metalli nei fungus ball e nei materiali endodontici testati è invece sovrapponibile è stata sovrapponibile tra il Fungus Ball e i materiali endodontici testati. Tale dato La concentrazione simile nei materiali endodontici e nel Fungus Ball suggerisce che il materiale endodontico svolga un ruolo nella patogenesi del Fungus Ball. Ciò suggerisce che i materiali endodontici inavvertitamente spinti nel seno mascellare durante i trattamenti canalari possano svolgere un ruolo fondamentale. I dentisti dovrebbero essere il più attenti possibile durante il trattamento di denti dell'arcata superiore per evitare di forare il pavimento del seno mascellare. e forse L'uso di materiali endodontici privi di zinco dovrebbe essere incoraggiato, essendo lo zinco un metallo che gioca un ruolo fondamentale nella crescita del fungo.

PAROLE CHIAVE: *Fungus ball mascellare • Materiali a base di ossido di zinco • Trattamento endodontico • ICP-MS (Inductively Coupled Plasma Mass Spectrometry)*

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Introduction

Continuity or contiguity of the upper teeth apical dental root foramina (molars and premolars, more rarely canines) with the maxillary sinus floor [1-3] may cause spread of odontogenic inflammatory and/or infective processes to the maxillary sinus. The most frequent infective process is the maxillary sinusitis and a less frequent form of possible odontogenic origin is a non-invasive mycotic form: fungus ball (FB) [4-7 5-8]. The most credible aetiology of FB is endodontic treatment of the upper teeth [2 8-12 2 4 9-12]. The time interval between endodontic therapy and diagnosis of FB is several years (6 on average) [11]. Usually, symptoms appear when the fungal mass fills the entire maxillary sinus. At this point, surgery becomes the only viable option.

It is well-known that endodontic sealers, long used in dental practice, are mostly made of zinc oxide, and it has been demonstrated [13 14] that zinc promotes fungal growth as it is an essential microelement for helping some fungi such as

Aspergillus sp. (which is the main pathogen leading to FB) survive and proliferate [5 7 6 8].
In a previous paper [13], we showed that eugenol and the mixture of powder-eugenol of endodontic sealers inhibit the growth of *Aspergillus*. Even after 75 days, eugenol has a slight inhibitory effect on such growth. The amount of zinc oxide in powders has a direct bearing on their effectiveness in promoting *Aspergillus* growth. Therefore, it is likely that endodontic sealer may cause the development of mycotic sinusitis as eugenol gradually loses its ability to inhibit the growth of *Aspergillus*, leaving zinc oxide, which is essential for the growth of *Aspergillus*.
Over a series of 102 patents with FB, clinical and radiologic diagnosis showed the presence of endodontically-treated teeth or post-extraction sites of previously endodontically-treated teeth in 99% of cases [9].
This is, however, only a clinical observation. Thus, is it sufficient to justify the odontogenic origin of FB? The objective of this prospective study was to evaluate the presence of zinc and other metals contained in the most important endodontic sealers in FB samples and in the adjacent mucosa in order to determine if there is a statistically significant difference.

Materials and methods

From October 2002 to September 2007, 53 patients affected by FB were surgically treated by endoscopic surgery at the Department of Otorhinolaryngology, University of Brescia (Italy). All patients were also examined in the Dental Clinic before undergoing surgical treatment. Demographics and historical data were collected along with radiographic findings (panoramic, intraoral periapical radiographs and CT of the sinuses).
The treatment consisted in the complete removal of the fungal debris with reaeration of the sinus through an endoscopic minimally-invasive approach [15 31]. During the surgery a sample of normal mucosa was taken from the maxillary sinus.
After the surgical procedure, FB and maxillary mucosa samples were sent to the Pathology Department to confirm diagnosis and to the Industrial Toxicology Laboratory of the Section of Occupational Medicine and Industrial Hygiene of the Department of Experimental and Applied Medicine to perform inductively coupled plasma mass spectrometry (ICP-MS).
Using the same method, the following endodontic materials were also examined in the Occupational Medicine Laboratory using the ICP-MS method [16 20]:
PCS-Pulp Canal Sealer (Kerr, Romulus - MI 48174, USA);
N2-Sargenti Cement (Hager & Werken GmbH & Co KG, Duisburg 47006, Germany);
Endometasone (Septodont, Saint-Maur-Des-Fossès, Cedex 94107, France)
Standardised gutta-percha (Kumapan, Dia Dent Group Internetional, Choongehong Buk Do. Korea)
Non-standardised gutta-percha (Dentsply Maillefer 1338 Ballaigues Switzerland).
Two samples of each material were analysed and the average value of each metal was kept as a reference.
Moreover, 5 cultures of different fungi (*Aspergillus fumigatus*, A*spergillus flavus, Aspergillus niger*, *Alternaria, bipolaris*) were analysed with the same methods.

Measuring instruments and procedures

Tissue specimens were oven dried at 70°C for 2 h, left to cool at room temperature in a desiccator, and finally weighed. Samples were then dissolved in HNO_3 (ACS reagent, Sigma) 70% (v/v) for 2 h at 70°C.
The digested samples (0.1 ml) were diluted with deionised water to 5 ml.
Prepared samples underwent ICP-MS analysis on a Perkin Elmer ELAN DRC II instrument (Perkin Elmer, Woodbridge, ON, Canada) using the analytical technique total quant with external calibration.
The method's accuracy was determined in natural water reference materials (NIST 1640, National Institute of Standard and Technology, Gaithersburg, MD).
The coefficients of variation (CV) ranged from 4 to 8% among series and from 6 to 12% between series. The instrument was calibrated using a standard solution at a concentration of 10 µg/l (Multielement ICP-MS Calibration Standard 3, Matrix per Volume: 5% HNO_3 per 100 ml, Perkin Elmer Plus).
The limits of detection (LOD) were determined on the basis of three standard deviations (SD) of the background signal, and the following values (as µg/l) were obtained: 0.006 for Bi and Ba; 0.005 for Hg; 0.004 for Cu, Sr and Pb; 0.003 for Ag; 0.002 for Zn and Mn [17 30].

Statistical analysis

The data are to be considered as average values (Standard Deviation, SD).The non-parametric Mann-Whitney test was used to compare metal (Zn, Cu) concentrations in the FB and maxillary mucosa. SPSS was used to analyse the data. A $p < 0.005$ was considered as statistically significant.

Results

Anamnestic and radiographic data showed that 99% of patients had undergone at least one endodontic treatment in the maxillary sector where the FB was found. The results of ICP-MS of elements in endodontic materials are listed in Table I. Since zinc and copper are widely present in endodontic materials, they may be used as a reliable finger-printing of those materials. In Table II, the results of FB, mucosa and cultures of fungi are compared to endodontic material.
It can be clearly seen that zinc and copper are the elements most widely used in endodontic materials. These metals were considered in statistical analysis to sustain the evi-

Table I. Average values by ICP-MS of elements in endodontic sealers.

Metals in endodontic sealers									
Metal [μg/g]	**Cu**	**Zn**	**Pb**	**Ba**	**Mn**	**Ag**	**Bi**	**Sr**	**Hg**
Endometasone	50	395000	17500	22500	3	4,5	35	160	8
N2	75	620500	8	0	1.5	160	193000	0	0
PCS	70	601000	15	25	7.5	421000	10	8.5	45
Non-standardised guttaperca	115	719500	5	2	0	1	0	0.1	0
Standardised guttaperca	100	627500	5	1600	7	2.5	0	30	0

Cu: copper, Zn: zinc, Pb: lead, Ba: barium, Mn: manganese, Ag: silver, Bi: bismuth, Sr: strontium, Hg: mercury.

dence for the presence of endodontic materials in FB samples compared to their absence in normal mucosa.
Various metals may be traced [18 21] in all biological tissues, but this objective of this study was to show a difference between the amounts of zinc and copperin the FB (and therefore of exogenous origin) and that of those to be found in mucosa.
FBs were found to contain a quantity of zinc and copper that was more similar to endodontic materials tested than the mucosa samples, while lower average values were detected in fungi cultures.
The Mann-Whitney test is significant with $p < 0.05$ for metals tested comparing samples of FB and mucosa (Table II).

Discussion

ICP-MS is a highly sensitive, multielement analytical method that has increasingly become affordable to many labs. It is being applied to biological samples and has several advantages: multielement stimulation determinations, excellent detection limits, a wide linear dynamic range and a high sample throughput [22 18].
The results obtained with ICP-MS and statistical analysis show that metals present in the most widely used endodontic materials are found in significantly higher amounts in FBs compared to mucosa.
Traces of these metals, as found in nasal mucosa, are however compatible with those found in lung tissues and in the upper respiratory tract as a result of atmospheric contamination by microelements such as lead, zinc and barium [20 22]<?>

Conclusions

FB is a non-invasive mycotic sinusitis. While the incidence of FB is not well known (3.7%) [21 23], it is certainly of substantial relevance as it cannot be treated through medical therapy. Therefore, a surgical procedure is needed [23 24 32 33], which represents an invasive intervention for the patient.
The correlation between endodontic therapies and FB as shown in a case control study [9] and supported by different case series [2 5 11 25 2 6 11 26], together with the demonstration, through this study, of the presence of metal contents in endodontic material in samples of FB, contributes to strongly support the odontogenic aetiologic hypothesis. Moreover, Tung-Lung Tsai et al. [26 27] have demonstrated that there is no relation between obstruction of the ostiomeatal complex and FB, thus confirming a different aetiopathogenesis of FB.
Despite the fact that research is progressing in this direction, it is crucial that dentists take some simple precautions to prevent this problem from occurring. It is advisable to execute root canal therapies on antral teeth (molars, premolars, canines of upper jaw) with the utmost attention to work length (electronic apex locator and intraoral periapical radiographs [27 28]) to avoid over-instrumentation, to use warm canal obturation techniques which need low amounts of sealers and possibly endodontic sealers not based on zinc oxide [28 29]. The follow-up is present beyond the apex after endodontic treatment should include a radiological check-up and evaluation of possible symptoms at least once a year in order to precociously identify any pathologic signs and to subsequently refer the patient to an ENT specialist.

Table II. Average values and SD of ICP-MS for normal mucosa, fungus ball, endodontic materials and a culture of *Aspergillus* spores and Mann-Whitney test Results.

Metal [μg/g]	Cu	Zn
Endodontic sealers	82.00 (25.64)	592700 (119599)
Fungus ball	98.84 (128.02)	3199 (15961.58)
Mucosa	15.73 (26.09)	220.75 (965.91)
Fungl	7.8 (5.11)	7.37 (3.97)
Fungus ball vs. mucosa	p = 0.000	p = 0.000

Cu = copper, Zn = zinc.

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