Abstract
The electromyography has been widely used as a diagnostic method for facial muscle conditions. The aim of this study was to determine the RMS (Root Mean Square) value of the electromyographic signal of facial muscles in children with normal occlusion or cross-bite as well as to verify a possible clinical application of this parameter. “Normal group” was composed of female children with normal occlusion and “cross-bite group” was composed of female children with cross-bite. We performed quantitative electromyographic analysis of masseter and temporal muscles at rest and with maximum biting force. Our results showed that the occlusion modifications affect normal masticatory muscle function, and that the quantitative electromyography is an important tool to help diagnosing the malocclusion in female children. Considering the analysis of the results, we verified that the electromyographic rest activity of the masseter or temporal muscles in cross-bite female children is higher in relation to the rest activity of normal occlusion female children. We concluded that the RMS value of the masseteric and temporal electromyographic signal has a diagnostic value to discriminate female children with cross-bite from those with normal occlusion.

Keywords: Electromyography, Malocclusion, Cross-bite.

Quantitative electromyographic assessment of facial muscles in cross-bite female children
Avaliação eletromiográfica quantitativa de músculos faciais em crianças do sexo feminino com mordida cruzada

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http://dx.doi.org/10.4322/rbeb.2012.054
Introduction
Electromyography consists of the graphic registration of the myoelectric signal, and it represents an important tool for quantification of muscular activity at rest and during contraction (Basmajian and De Luca, 1985; Fridlund and Cacioppo, 1986). Thus, electromyographic analysis has been widely used as a diagnostic method for muscular conditions in several situations and in different muscle functions. For example, as for the dental research, electromyographic analysis is used to measure the electric activity of normal facial muscles, or in pathological conditions (Basmajian and De Luca, 1985; Fridlund and Cacioppo, 1986). Among the pathological conditions are the temporomandibular dysfunctions, characterized by extrinsic or intrinsic alterations in the temporomandibular joint, which lead to functional disorders in the muscles of the face – mainly masseter and temporal muscles (Ali et al., 2003; Castroflorio et al., 2004; Dahlström, 1989; De-Luca, 1997; Mohl et al., 1990a).

It is well known that different etiologies in adults and children can lead to occlusion disorders in which the diagnosis may or may not be supported by electromyography (Garcia-Morales et al., 2003; Gomes et al., 1998; Mohl et al., 1990b; Palomari-Tobo et al., 1996; Shirrazi et al., 1990). For example, Sgobbi de Faria and Bézin (1998) showed that the temporal resting activity in adults is higher than the masseter resting activity, even though normal subjects do not show excessive activity of those muscles in mandibular resting position. Thus, parameters obtained from the electromyographic signal processing, such as amplitude or the root mean square value (RMS), may have relevant diagnostic value.

One important pathological type of occlusion that can be evaluated by electromyography in children is cross-bite. Cross-bite is the term used to indicate an abnormal buccolingual relationship of the teeth and it is a potential risk of temporomandibular disorder (Egermark et al., 2003). Subjects with cross-bite exhibit larger muscular tension patterns at rest position if compared with normal occlusion subjects (Li et al., 1994; Miyamoto et al., 1999; Nuño-Licona et al., 1993; Rodrigues et al., 2006; Thilander et al., 2002). Several authors also indicate that unilateral cross-bite in children can lead to alterations in masseteric and temporal tension mainly when it is associated with jaw deviation.

Considering the aforementioned, it is reasonable to consider that the RMS value of the electromyographic signal, which is an electromyographic parameter related to muscle activity, would help to study and distinguish levels of masseteric and temporal activity in children separated in groups by age, sex, and type of occlusion, including cross-bite. Thus, in order to contribute to this kind of study, we specifically aimed to determine RMS value of the electromyographic signal of facial muscles in female children with normal occlusion or with cross-bite by trying to verify a possible clinical application of this parameter.

Materials and Methods
Subjects
Female children with normal occlusion (Class I) were included in the “Normal Group” (N = 11) whereas female children diagnosed with cross-bite without considering the specific classifications for these occlusion dysfunctions were included in the “Cross-Bite Group” (N = 13). Both groups were composed of female children between 7 and 11 years old, taking into account their availability to participate on this study. The mean age in the Normal Group was of 10 ± 1.2 years, whereas in the Cross-Bite Group, the mean age was 9 ± 1.3 years.

The diagnoses and types of occlusions were provided by specialized orthodontists and dental-surgeons from the Dentistry Clinic of UMC (Universidade de Mogi das Cruzes). The specific procedures were intra-oral clinical evaluation, dental models, and X-rays to visualize the relation between the teeth, maxillaries and temporomandibular joints. Normal occlusion was considered the normal antero-posterior relation between the two dental arches (Peres et al., 2002) and cross-bite was considered the abnormal buccolingual relation of the teeth (Almeida, 1997).

All procedures with voluntary patients were approved by the Human Research Ethics Committee at UMC.

Instrumentation
The acquisition and processing of the masseter and temporalis myoelectric signal (Figure 1) were performed with an electromyographic system composed by the following devices: a six-channel bioelectric amplifier, which has been developed at the Laboratory of Biomedical Instrumentation of the Nucleus of Technological Research at UMC specific for the purpose of this study; a DC power source (Hewlett Packard E 3631) which supplied the amplifier; a true RMS digital multimeter (Tektronix DMM 916); an oscilloscope (Tektronix TDS 210); a 3-way shielded cable; Ag/AgCl surface electrodes and an isolator transformer specific for medical purposes (Isobox 600, Toroid do Brasil), which isolates the DC power source and the oscilloscope from the power line.
Each channel of the amplifier (Figure 2) displayed certain characteristics such as: an on/off switch, off-set adjustment, adjustable gains varying from 1,000 and 50,000-fold, a differential input, and a BNC type output. The bandwidth was adjusted through a high pass filter of 8 Hz, a 60 Hz-notch filter, and a low pass filter of 10 kHz to minimize interference of low frequency noise that originates from unwanted movement of the cables and electrodes, and high frequency noise that originates from several sources (bandwidth ranging from 8 to 10,000 Hz; Figure 3). Those filters were all based on the integrated circuit TL074. The notch filter was used to decrease the incoming signal from the electric net (Fridlund and Cacioppo, 1986).

The amplifying circuit was an INA-101 type with a high common rejection rate of (106 dB at 60 Hz) and high input impedance (10^10 Ω). The amplifier input impedance, CMRR and maximum peak-to-peak noise were, respectively, 10 MΩ, 48 dB (at 150 Hz) and 1 µV. The channels of the amplifier were power supplied with ± 9 V (isolated DC power source mentioned before).

The devices used for the exams (Figure 2) functioned so that the myoelectric signals during rest and maximum voluntary contraction were captured by the surface electrodes and transmitted to one of the channels of the amplifier via the 3-way shielded cable connected to the electrodes. The myoelectric signal was pre-amplified (gain of 10-fold in INA-101), filtered by (8 Hz) in sequence with a gain selection stage (1,000 to 50,000-fold), and with the low pass bandwidth filter (10 kHz). Then the signal was filtered in the 60 Hz-notch filter and, finally, the signal was applied to a buffer so it could have power enough to be simultaneously displayed on the oscilloscope and measured on the multimeter. With this procedure, the RMS value of the myoelectric activity was displayed on the multimeter, and the graphic registration displayed on the oscilloscope screen (see Figure 1).

All the equipments connected to the power line through the isolator transformer (oscilloscope and DC power supplier) were also connected to a ground wire.

The surface electrodes used for recording the myoelectric signal were 0.5 mm thick and 5 mm radius. Those were very light electrodes, weighing 500 mg, with an appropriate size for the requested position, independent of the format or size of the face. The electrodes had 10.5 mm long perpendicular wires that were connected to the cable endings.

The electrodes were made of pure silver (Ag) and were submitted to immersion in Sodium Chloride, thus becoming silver-silver chloride electrodes (Ag/AgCl). As previously indicated for electromyographic recording, a small layer of conductive gel was applied to the surface of the electrodes in contact with the skin, which helped reducing the impedance and, therefore, increased the signal fidelity (Fridlund and Cacioppo, 1986).

Some of the characteristics of the devices used for the recording of the electromyographic signal, such as filter cut-off frequency, amplifier gain, the mate-
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Figure 2. Electrical diagram of one channel of the amplifier. The characteristics of the circuit are described in the text.

Figure 3. Amplifier bandwidth ranging from 8 Hz to 10,000 Hz and including the effect of the 60 Hz-notch filter.

Positioning of the electrodes
The positioning of the electrodes in the masseter muscle was determined by palpation of the zygomatic bone and inferior angle of the jaw, with the objective of determining the origin and muscular insertion, as
well the direction of the fibers. Soon after, a palpation of the muscle in contraction was performed, identifying the area of larger mass or muscular belly (Sgobbi de Faria and Bérsin, 1998).

Initially, the positioning of the electrodes in the temporal muscle occurred with the palpation of the anterior portion while the patient voluntarily contracted those muscles. Soon after, a point to 50 mm of the tragus was marked on his face. The first electrode was positioned 20 mm vertically above this point and the second electrode was positioned 20 mm from the first electrode and toward the muscle fibers. The reference electrode was positioned in the superior and central area of the frontal bone of the subjects (De-Luca, 1997).

Procedures
The exams were performed in the masseter and temporal muscles of subjects of Normal or Cross-Bite Groups. The subjects were seated in a dental chair with their heads supported and with a Frankfurt plane parallel to the ground.

According to the laws of the Human Research Ethics Committee of UMC, the patients and parents were fully informed regarding the procedure to be conducted, and signed the consent agreement before beginning the tests.

Initially, we cleaned each subject’s face skin with cotton embedded with hydrated ethyl alcohol for removal of excess oil and died skin cells. Afterwards, the electrodes were positioned and fixed with non-allergic adhesive tape.

After the electrodes were positioned, we verified the occurrence of noises in the amplifier input by connecting the ends of the 3-way cable to create a short circuit. The amplifier gain was set in 1,000-fold. In this condition, the RMS value of the signal on the amplifier input was acceptable if it was less than 0.1 µV (0.1 mV in the multimeter). Once concluded, we undid the short circuit of the input amplifier and connected the 3 way cable to the electrodes that were already positioned on the patient’s face.

Measurement of RMS at rest and during maximum biting
The measurements of the rest RMS values (RMS<sub>R</sub>) of EMG, for both masseter and temporal muscles were performed, not simultaneously, in the following sequence: once the input cable of the amplifier was fixed on the electrodes placed on the patient’s face, the subject was instructed to stay totally immobile until the RMS<sub>R</sub> value displayed by the multimeter could stabilize. We took note of 5 RMS<sub>R</sub> values after an interval of 25 seconds from the electrodes positioning.

The measurement of the maximum biting RMS (RMS<sub>M</sub>) was performed, not simultaneously, in the following way: soon after the measurement of the RMS<sub>R</sub>, the patient was instructed to bite with maximum force. During this condition, we wrote down 5 values of RMS<sub>M</sub> for each muscle at a time.

The measurements in both conditions (at rest or maximum biting) were made three times (1 minute interval) in each muscle. With this procedure, we obtained 15 rest values and 15 maximal biting values for each tested muscle (left and right masseter or temporal muscle). The mean of these values obtained in a certain condition was considered as being the RMS value of the muscle in that condition and on that face side.

Additionally, the means of RMS of left and right muscles taken altogether were considered as being the bilateral RMS value of that muscle.

Analysis of the data
All groups of data presented normal distribution. Therefore, parametric statistic could be performed. The masseter or temporal RMS means of the left and right side of the two analyzed groups was compared at rest situation (R) or at maximum biting situation (MB) using the unpaired Student t-test (independent groups). The ratio between RMS<sub>M</sub> and RMS<sub>R</sub> (MB/Rest) was calculated for every subject. The MB/Rest means were calculated for the masseter and temporal muscles in the two analyzed groups. We used the unpaired Student t-test (independent groups) to compare children with normal occlusion to those with cross-bite. The values are expressed as mean ± standard deviation, and the statistical significance was considered for p < 0.05.

Results
Table 1 summarizes the electromyographic values obtained in masseter and temporal muscles of female children with normal and cross-bite occlusion.

The RMS<sub>R</sub> mean values for the left masseter were not statistically different from the RMS<sub>R</sub> mean values for the right masseter. As for the RMS<sub>M</sub> of the temporal muscles, the results obtained also showed no statistical difference between left and right muscles. Accordingly, the mean values of RMS<sub>M</sub> of the left masseter (or temporal) muscle was not statistically different from the mean values of RMS<sub>M</sub> of the right masseter (or temporal) muscle. Therefore, bilateral RMS
values were taken together to calculate the means (N = 22 for normal group and N = 26 for cross-bite group).

Figure 4 shows the bilateral masseteric RMS_R means of patients from normal and cross-bite groups. The mean value in normal group (1.8 ± 0.1 µV) is statistically different from that in cross-bite group (2.6 ± 0.1 µV; p < 0.05).

Figure 5 shows the bilateral masseteric RMS_M means of patients from normal and cross-bite groups. The mean value in normal group (170 ± 25 µV) is not statistically different from that in cross-bite group (151 ± 9 µV; p > 0.05).

Figure 6 shows the bilateral temporal RMS_R of patients from normal and cross-bite groups. The mean value in normal group (2.4 ± 0.2 µV) is statistically different from that in cross-bite group (3 ± 0.2 µV; p < 0.05).

Figure 7 shows the bilateral temporal RMS_M means of patients from normal and cross-bite groups. The mean value in normal group (124 ± 15 µV) is not statistically different from that in cross-bite group (120 ± 6 µV; p > 0.05).

Table 1. Electromyographic values (mean ± standard deviation) of masseter and temporal muscles (right and left sides) of female children (normal and cross-bite) in Rest and in Maximal Biting – MB.

<table>
<thead>
<tr>
<th>Type of occlusion</th>
<th>Left side</th>
<th>Right side</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Masseter</td>
<td>Temporal</td>
</tr>
<tr>
<td></td>
<td>Rest</td>
<td>MB</td>
</tr>
<tr>
<td>Normal (µV)</td>
<td>2 ± 0.2</td>
<td>138 ± 25</td>
</tr>
<tr>
<td>Cross-bite (µV)</td>
<td>2.5 ± 0.2</td>
<td>139 ± 11</td>
</tr>
<tr>
<td></td>
<td>1.7 ± 0.2</td>
<td>190 ± 34</td>
</tr>
<tr>
<td></td>
<td>2.7 ± 0.2</td>
<td>164 ± 13</td>
</tr>
</tbody>
</table>

Figure 4. RMS voltage (µV) means obtained of masseter of female children at rest condition. The voltage RMS mean of normal group (1.8 ± 0.1) is statistically different from that in cross-bite group (2.6 ± 0.1; p < 0.05).

Figure 5. RMS voltage (µV) means obtained of masseter of female children in maximal biting condition. The mean of normal group (170 ± 25) is not statistically different from that in cross-bite group (151 ± 9; p > 0.05).

Figure 6. RMS voltage (µV) means obtained of temporal of female children at rest condition. The mean of normal group (2.4 ± 0.2) is statistically different from that in cross-bite group (3 ± 0.2; p < 0.05).

Figure 7. RMS voltage (µV) means obtained of temporal of female children in maximal biting condition. The mean of normal group (124 ± 15) is not statistically different from that in cross-bite group (120 ± 6; p > 0.05).
The ratio between $RMS_{M}/RMS_{R}$ means for temporal muscle of female children in the two studied groups are shown in Figure 9. The mean value in normal group (52 ± 5) is not statistically different from that in cross-bite group (47 ± 4; $p > 0.05$).

**Figure 8.** Ratio between RMS voltage means in the condition of maximal biting and rest ($MB/Rest$) obtained of the masseter muscle of female children in the two studied groups. The $MB/Rest$ mean of normal group (95 ± 13) is statistically* different from that in cross-bite group (61 ± 4; $p < 0.05$).

**Figure 9.** Ratio between RMS means in the condition of maximal biting ($MB/Rest$) and rest obtained of the temporal muscle of female children in the two studied groups. The ($MB/Rest$) mean of normal group (52 ± 5) is not statistically different from that in cross-bite group (47 ± 4; $p > 0.05$).

**Discussion**

In this research we have performed quantitative electromyographic study of facial muscles of female children with normal occlusion or with cross-bite. In this study we verified that cross-biting significantly influences the natural function of the masticatory musculature. This result, as a whole, corroborates findings of other authors who showed that occlusion alterations can affect masticatory function and that children exhibit a correlation between posterior cross-bite and temporomandibular dysfunction – TMD (Rodrigues et al., 2006; Thilander et al., 2002). Our results give quantitative data supporting the idea that cross-bite orthodontic treatment could result in temporal and masseter function improvements, just like observed by Rodrigues et al. (2006). Furthermore, the information supplied by electromyography can be used to diagnose and treat subjects with TMD, as well as to analyze muscles of the jaw when considering age, sex, weight and skeletal types of the population (Moses, 1995; Pinho et al., 2000). In this sense, it has been shown, in relation to what occurs with children, that masseteric activation is more evident than temporal activation in adults (Pancherz, 1980). Therefore, those considerations support our decision of assuming the inclusion of children’s groups with different patterns of cross-bite as the only groups to be evaluated at this time.

In a work performed by Li et al. (1994) it was concluded that a larger muscular activity was found in children with cross-bite at rest position which, in turn, is consistent with those obtained in this work. We have found larger electromyographic activity at rest of the masseter muscle in patients with cross-bite in relation to those with normal occlusion (see Figures 6 and 7).

In another work performed by Gavião et al. (2001), the masticatory efficiency of children with normal primary occlusion and malocclusion was evaluated and correlated to anatomical variables. A total of 30 children were divided into 3 groups, with group I being the normal occlusion, group II with posterior cross-bite, and group III with open bite. The subjects bit tablets of standardized silicone, however, group 1 fragmented the tablets in a larger number of smaller pieces than those of groups 2 and 3. The authors concluded that the occlusion is a factor that influences the mastication process. In our study, we supposed that it would be reasonable to find significant differences in the masseteric or temporal RMS at the maximum biting condition of children with cross-bite in relation to those with normal occlusion. However, our findings related to maximum biting did not confirm experimentally what we have supposed previously and also were different from Gavião et al. (2001). This might be due to the fact that we had only female children in our study. Therefore, further studies specifically directed to sort this out are still necessary.

Additionally, Lowe and Takada (1984), working with children presenting normal occlusion or malocclusion, investigated the activity of facial muscles.
They related TMD with the increase of muscular tension at rest and with a loss of force. This relationship was relevant for the increase of masticatory muscle activity at rest while decreasing during maximum biting in the asymptomatic patients when compared to the asymptomatic ones (Dahlofström, 1989; Mohl et al., 1990b; Oliveira et al., 2004). Subjects with cross-bite also demonstrated larger patterns of muscular tension than of that in individuals with normal occlusion (Li et al., 1994; Miyamoto et al., 1999; Nuño-Licona et al., 1993). Such findings are partially corroborated by our results, mainly with respect to the masseter at rest (see Figure 2).

It is well established now that the RMS of the electromyographic signal presents a linear relationship with the force of contraction of the masseter and anterior temporal muscles (Buxbaum et al., 1996; Dahlofström, 1989; De-Luca, 2003; Fridlund and Cacioppo, 1986; Pancherz, 1980; Wang et al., 2000). That finding emphasizes the importance of this parameter as a diagnostic tool for pathological muscle mass evaluation. Considering the aforementioned, Alvarez (2004) studied normal young adult and TMDs subjects, and demonstrated the effective diagnostic value of this parameter for this dysfunction regarding the relationship of RMS with masseteric and temporal force. That work pointed out that the RMS value of the electromyographic signal would be a parameter worthwhile for the diagnosis of other dysfunctions. In accordance, we have shown that this parameter could be useful to help diagnosing female children with cross-bite.

In our final analysis, we concluded that quantitative electromyography is a useful tool to aid in the diagnosis of occlusal changes in female children. Furthermore, the RMS value of the electromyographic signal of the masseter and temporal muscles at resting has a potential diagnostic value to clinically discriminate children with cross-bite in relation to those with normal occlusion.

Acknowledgements
To Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) and Fundação de Amparo ao Ensino e Pesquisa (FAEP-UMC).

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