The effect of NitrAdine on the *Candida* levels of maxillary removable appliances

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Objective: *Candida* colonization is a consequence of orthodontic treatment and can lead to oral candidosis as a complication of maxillary removable appliance treatment. During orthodontic treatment, it is important to minimize colonization to prevent active infection that could consequently interfere with treatment. Hygiene is the most important factor in managing colonization; in this study, the efficacy of NitrAdine to reduce *Candida* was tested. **Method and Materials:** A randomized, double-blind, placebo-controlled trial was performed. Ninety-two patients 11 to 14 years of age were recruited at the Children's and the University Dental Clinics at Mater Dei Hospital, Tal-Qroqq, Msida, Malta. Forty-four patients used the product with NitrAdine, while 48 patients used a placebo. Sampling employing the imprint technique was performed before and after the product was used. Brilliance *Candida* agar was used for cultures and identification. Further identification was performed using Auxacolor 2 when required. **Results:** The control group had a statistically significant increase in *Candida* during treatment, while the experimental group had a nonstatistically significant decrease. **Conclusion:** It was concluded that NitrAdine may reduce the *Candida* burden in maxillary removable appliances. Larger sample sizes are needed to achieve statistical significance. *(Quintessence Int 2012;43:239–245)*

Key words: Candida, maxillary removable orthodontic appliances, oral candidosis, oral hygiene

Candida species routinely colonize the oral cavity and form part of the normal commensal flora. Maxillary removable appliance treatment is known to predispose patients to oral *Candida* colonization since the removable appliances provide a niche for *Candida* to thrive.[1,2] This is brought about by various factors including the challenge of effective oral hygiene (including the hygiene of the maxillary removable appliance). *Candida* colonization is the first step toward oral candidosis, which is a potential complication of maxillary removable appliance use. This may interfere with orthodontic therapy and lead to more severe complications such as systemic candidosis.[2] Infection is generally treated with antifungal agents, which include polyenes and azoles. Although polyene resistance is rare, high minimum inhibitory concentration (MIC) to azoles is inherent to some *Candida* species, including the commonly isolated species *C glabrata* and *C krusei*. *C albicans* is also known to acquire resistance to azoles. Moreover, *Candida* in the mouth is generally present as biofilm, which is associated with high MICs to antifungal agents.[3]

Different techniques employed to isolate *Candida* from the oral cavity to measure prevalence, such as salivary specimens, impression cultures, and imprint cultures, differ in their results. The tongue seems to be the main oral reservoir of *Candida*.[4] *C albicans* is the most predominant isolate colonizing the oral mucosa.[5] To successfully colonize the mouth, *C albicans* is able to adhere to surfaces, grow, change forms, and evade host clearance mechanisms.

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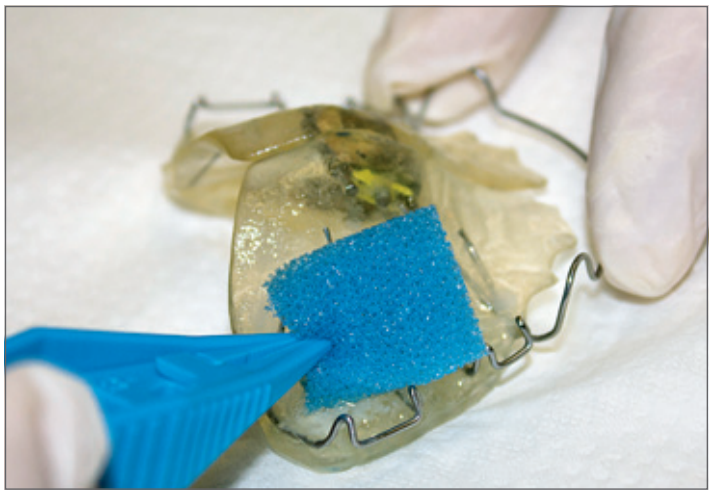

Fig 1 The imprint technique employed in this study.

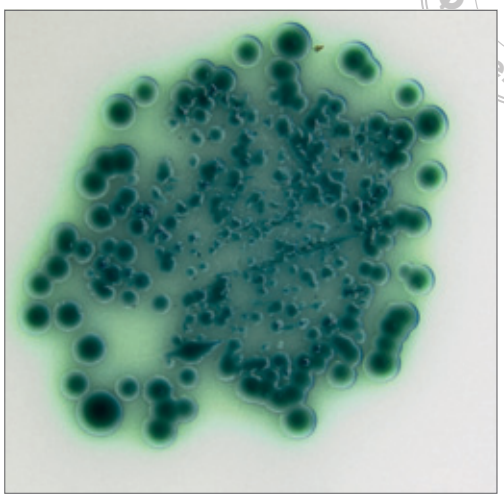

Fig 2 *C albicans* on Brilliance *Candida* agar inoculated by the imprint technique.

These characteristics can be considered virulence factors.[6] The step from colonization of the oral mucosa to infection depends on virulence factors, environmental factors (such as prostheses and orthodontic appliances), and host defense factors. These are not independent of each other, and understanding the transformation from commensal to pathogen is still a challenge. It is understood that a fine balance exists, and slight changes may easily tip the balance with infection taking place.[7] Oral hygiene plays an important role in the prevention and treatment of *Candida*-related complications in oral medical device wearers. In these individuals, oral hygiene includes the hygiene of the device. Inflammatory conditions in these patients are related to *Candida* biofilm on the device, and control is therefore crucial in management of inflammation. Lack of hygiene allows the formation of plaque (biofilm), and accumulation of food particles further encourages growth of microorganisms including *Candida*. Effective sanitization of maxillary removable appliances in children has led to amelioration of signs and symptoms of inflammation.[1] Effective sanitization can therefore minimize the need for antifungal therapy, which in turn will minimize the risk of emergent antifungal drug resistance.[3]

NitrAdine (Medical Interporous, MSI Laboratories) is a disinfecting formula for the care and hygiene of oral medical devices. The ingredients are patented; however, the product acts by oxidization. In the study, we evaluated the efficacy of this product at reducing the *Candida* carriage.

METHOD AND MATERIALS

Ethical approval was obtained from the University Research Ethics Committee of The University of Malta, Tal-Qroqq, Malta (reference no. 51/2008). This approval ensures that the study is in accordance with the Helsinki Declaration. All patient's parents or guardians (since patients were all younger than 18 years of age) signed a consent form and received a study information sheet.

Patients

Recruitment was performed at the Children's and University Dental Clinics of Mater Dei Hospital, Tal-Qroqq, Msida, Malta, between January and December 2009. To be fit for the study, patients had to have attained their 11th but not yet their 15th birthday. The patients had to be in constant use of a

maxillary removable orthodontic appliance for more than 1 month. Significant tooth movement (1 mm a month), the ability to speak clearly with the appliance, and signs of wear evident on the appliance were the criteria used for compliance. The same criteria were used to measure compliance after treatment with the product.

Patients with conditions or undergoing treatment that could predispose them to oral candidosis, including immunologic disorders, diabetes, steroid therapy, and antibiotic therapy, in the 3 months prior were also excluded. Patients who underwent antifungal therapy in the previous 3 months were also excluded. However, patients receiving asthma medication containing small amounts of steroids were not excluded.

Study design

A randomized, double-blind, placebo-controlled design was adopted for the study. The patients were given a package with a serial number. This number also acted as a patient identification number. The key to the serial number was held by the assigning investigator, who was not involved in the selection of patients. The order of the list randomly assigned each consecutive suitable patient to either the placebo or experimental group. On recruitment, the assigning investigator progressed down the list, instructing the operating investigator which number package to use and adding the details of the patient to the list. The operating investigator labeled all samples collected from the patient with this serial number. The sample size was calculated using a previous study as a model.[8] The sample size was obtained at 95% level of significance and 80% power. According to the previous study, the *Candida* carriage rate was approximately 58%. The sample size was calculated to detect a drop of 30% following the use of the product. This resulted in a sample size of 48 in each group. To allow for dropouts, 120 patients were recruited in all. The previous study was performed between January and June 2008. No patients from the previous study were recruited for the current study.

The product

Placebo and experimental packages and tablets had the same appearance. The placebo was made of the same ingredients as the product, excluding the active ingredients. Forty-two tablets were packaged and given a serial number by the assigning investigator. This was enough for 6 weeks of usage. The patients were instructed to use the product once daily. They were told to remove the appliance and brush it with a toothbrush under running water without using any other product. They were then asked to put the appliance in a glass of lukewarm water, add one of the provided tablets, and leave the appliance in the solution for 20 minutes. The appliance could then be rinsed well and worn again.

Sampling

Sampling was performed by the operating investigator before and after treatment with the product using the imprint technique (Fig 1) as described by Arendorf and Walker.[4] The foam pads were 1 × 1 cm, and the imprint was taken from the flattest area of the palatal side of the appliance. Brilliance *Candida* agar (Oxoid) was used for primary isolation of *Candida* (Fig 2).

Candida counts and identification

The primary media were incubated for up to 72 hours at 30°C after inoculation. Any growth of yeasts was counted. If more than one type of yeast was cultured, the total count and count of individual species was taken. Any unidentified colonies were identified to the species level using Auxacolor 2 (Bio-Rad) and the Dalmau method using cornmeal agar (Oxoid) with Tween 80 (BDH Laboratory Supplies). Any yeast not identified as *Candida* species was ignored.

Statistical analysis

The Mann-Whitney *U* test was used to compare the populations of the two arms of the study. The Wilcoxon rank sum test was used to compare the pretreatment data with the posttreatment data. The analysis of variance regression model was used to see whether the sexes had differences between them with regard to fungal colonization.

Table 1	Distribution of colonization for the two groups				
Group	No. of patients free of *Candida* colonization throughout the study (%)	No. of patients colonized before product treatment but not after(%)	No. of patients colonized during treatment with product (%)	No. of patients colonized throughout the study (%)	Total
A	26 (54.2)	1 (2.0)	6 (12.5)	15 (31.2)	48
B	22 (50.0)	6 (13.6)	4 (9.1)	12 (27.3)	44

Table 2	Summary of the data with regard to the *Candida* species isolated in the study								
	No growth	*C albicans*	*C krusei*	*C parapsilosis*	*C tropicalis*	*C albicans C famata*	*C albicans C glabrata*	*C albicans C tropicalis*	Total
Before									
A	32	14	0	1	1	0	0	0	48
B	26	15	1	0	0	0	1	1	44
After									
A	27	18	0	1	1	1	0	0	48
B	28	14	0	0	0	0	2	0	44

RESULTS

The total number of patients recruited for the study was 120. Of these, 8 patients damaged their maxillary removable appliance, 6 were not compliant with the use of the product, 1 lost the maxillary removable appliance, 4 were lost to follow-up, 8 voluntarily dropped out of the study, and 1 had a high count of *Trichosporon mucoides* and was therefore excluded. Therefore, the number of patients considered suitable for the study until its completion was 92. When all the sampling and testing was complete, the assigning investigator divided the patients into two groups (A and B) but did not reveal which was the control and which was the experimental group. Group A was made up of 48 patients, of which 22 (45.8%) were boys and 26 (54.2%) were girls, while group B was made up of 44 patients, of which 17 (38.6%) were boys and 27 (61.4%) were girls. Therefore, boys made up a total of 42.4% vs 57.6% girls.

In group A, 26 patients (54.2%) remained free of *Candida* colonization, 1 (2.0%) was colonized before but not after NitrAdine treatment, 6 (12.5%) became colonized during the NitrAdine treatment period, and 15 (31.2%) were colonized throughout the study. In group B, 22 patients (50.0%) remained free of *Candida* colonization, 6 (13.6%) were colonized before but not after NitrAdine treatment, 4 (9.1%) became colonized during NitrAdine treatment, and 12 (27.3%) were colonized throughout the study. This means that in group A, 56.3% were not colonized by *Candida* after treatment with the product, while in group B, the percentage of patients not colonized was 63.6%. The data collected is summarized in Tables 1 and 2.

For the purpose of this exercise, the total count of organisms was taken for those patients having more than one species. The data were not normally distributed; therefore, nonparametric tests were used.

A *P* value of .299 indicates that the difference in colonization between groups A and B before NitrAdine treatment with the product was not statistically significant; therefore, the two groups before treatment were similar.

The NitrAdine pretreatment data in group A were compared with the posttreatment data. A *P* value of .008 indicates that the difference in the counts from before and after NitrAdine treatment with the product was statistically significant; therefore, the increase in the *Candida* counts during treatment was statistically significant.

The NitrAdine pretreatment data in group B were then compared with the posttreatment data. A *P* value of .353 indicates that the difference in the counts from before and after NitrAdine treatment with the product was not statistically significant; therefore, the decrease in the *Candida* counts during treatment was not statistically significant.

After the statistical analysis, the study was unblinded by the assigning investigator. Group A was the control group, while group B was the experimental group.

It was noted from the NitrAdine pretreatment data that girls tended to have higher *Candida* counts and also tended to be more colonized than boys. In fact, 22 girls (41.5%) were colonized vs only 11 (28.2%) boys. The mean count for all girls was 38 colony-forming units (CFUs), while that for the boys was nearly 19 CFUs. Due to these observations, it was decided to test if these differences were statistically significant. A *P* value of .098 indicates that the difference in the counts between boys and girls was not statistically significant.

The NitrAdine posttreatment data was also used to make similar observations. The percentage of boys colonized was 28.2%, which is the same as that before treatment. The mean count rose from almost 19 to 26 CFUs. The girls colonized rose from 41.5% to 44.0%, and the mean count also rose from 38 to 46 CFUs.

DISCUSSION

NitrAdine is a product designed to control the biofilm buildup on maxillary removable appliances and therefore control *Candida*, the growth of which can lead to complications. The current study was therefore designed to test the efficacy of this product in reducing the amount of *Candida* on maxillary removable appliances. This product has been tested in vitro where the modified Robbins device was used to generate biofilms with organisms including *C albicans* on methyl methacrylate as a substrate. NitrAdine was shown to be effective at removing biofilm with a > 3 log reduction in *C albicans* after application of the product.[9] The need for in vivo testing was felt, since more factors may be involved. In this study, *Candida* counts showed a decreasing trend in the experimental group; however, this was not statistically significant. Twenty-two patients (50%) in the experimental group were not colonized on first sampling and remained free of *Candida* during treatment. However, this was not the case in the control group. The experimental group showed a trend, although not statistically significant, toward a reduction in *Candida* counts. This indicates that the active ingredient, although it does not eradicate the colonization, does have some activity against *Candida* biofilm since the counts did not go up as in the control group. It is possible that the maxillary removable appliance is recolonized from other parts of the mouth.

In a previous study,[8] with a similar design and the same product but using saliva specimens, 62 patients were recruited (31 in each arm). This study looked at different criteria, namely biofilm formation, odor levels, and *Candida* carriage. This study concluded that the experimental group had a statistically significant decrease in plaque on maxillary removable appliances and also a statistically significant decrease in odor. This study also divided the patients into two groups—namely, the ones with colonization but no oral candidosis (< 400 CFU *Candida* per mL of saliva) and the ones with counts associated with oral candidosis (> 400 CFU *Candida* per mL of saliva). The product with the active ingredient showed a trend in decreasing the *Candida* count from > 400 CFU/mL to < 400 CFU/mL, but this decrease was not statistically significant. This study had a smaller population size as the current study, and it was suggested that a larger population size was required. It was also argued that saliva specimens were not the ideal specimens since *Candida* can be cultured from other sites of the mouth.[8] Therefore, the current study used the imprint technique to culture the *Candida* of interest, ie, those found on the surface of the maxilla that comes in contact with the palate. This technique was found more useful and practical than collecting saliva. Apart from being more representative of what is happening on the maxillary removable appliance, it was easier for patients to participate in the study. The entire procedure

was less time-consuming, needed fewer consumables, and proved to be less costly. Other studies found the imprint technique to be superior to saliva sampling and other sampling techniques in other studies since it is more specific to the site of interest.[10–12] However, some argue that the technique is less sensitive than the oral rinse and saliva methods of sampling.[2,13] One drawback of the technique is that it does not differentiate between carriers and individuals with an actual infection.

It was noted that this technique yielded fewer species when compared with the preceding study using saliva specimens. This finding was also reported by Mahmoudabadi et al.[2] In the current study, the main species was *C albicans* as expected and was isolated in 65 of 71 positive cultures and made up 87% of the total yeasts isolated. This contrasts with 67% obtained from the preceding study. *C parapsilosis* was isolated twice, *C tropicalis* was isolated three times, *C krusei* was isolated once, *C glabrata* three times, and *C famata* once. Other species including *C zeylanoides*, *C lusitaniae*, and *C guilliermondii* were isolated in the preceding study but not in the current one. There were only five episodes with two species being isolated from the same specimen. Again, this contrasts with the preceding study using saliva as a specimen, since 11.3% of positive cases had two species as opposed to 7% in this study. Orthodontic appliances have been associated with multiple species in other studies.[2] The reason for a higher prevalence of *C albicans* in this study could be the fact that the sampling technique picked up *Candida* that was actually colonizing the surface of the maxillary removable appliance. *C albicans* is the yeast with the most potential to adhere and colonize such surfaces. Saliva specimens may yield organisms that may be transient in the mouth. These could be present in food and the immediate environment and could therefore end up in the mouth. It was however argued that *Candida* colonization and plaque formation in the niche developed by an appliance can predispose other areas in the mouth not associated with the appliance to become susceptible for colonization and plaque (biofilm) formation.[14] All the studies reviewed here have shown that *C albicans* is the predominant species encountered in the mouth of patients with yeast colonization and infection.

This study also revealed that girls may have higher carriage rates than boys at the age group recruited for this study. Although this was not statistically proven and a separate study using larger populations is needed to evaluate this, it may be worth noting. Any differences between the sexes could mean that there are other factors playing a role in colonization. With regard to other studies, Hägg et al[14] also found a non-statistically significant difference between the sexes, with females showing a trend to have a higher colonization incidence. This study had only 24 males and 26 females. Similar results were obtained in a local study in which patients wearing maxillary removable appliances were compared with controls (nonwearers). Here, girls tended to be more colonized than boys (12 girls vs 9 boys in the maxillary removable appliance group and 10 girls vs 8 boys in the control group). The age groups were the same as in the current study. These differences were not tested for statistical significance, but again showed a similar trend.[15] All these studies show that females may be more predisposed to *Candida* colonization than males. In general, the oral hygiene of adolescent girls is far superior to that of similarly aged boys, so the finding is surprising in this aspect (S. Camilleri, MDH, personal communication). This shows that other factors acting with or independently of maxillary removable appliances may play a role in colonization. Therefore, the maxillary removable appliances in this study may not have been the only predisposing factor for *Candida* colonization, with other variables such as hormones possibly playing a role. If that is the case, it would be worth testing whether there is any difference between males and females with regard to the effect of maxillary removable appliance disinfecting products such as that used in this study. Such a study would have to have a larger population of patients.

The power calculation before starting this project was based on a study using saliva specimens. The imprint technique gave a much lower proportion of subjects

with a positive culture on first sampling. In fact, 63% were culture-negative; therefore, in any future studies, the sample size should be more than double the size used in this study to achieve significance. A study on a larger scale will also make it possible to evaluate any possible difference in *Candida* carriage and maxillary removable appliace use between males and females as well as any differences with regard to the action of the product.

CONCLUSION

NitrAdine has a beneficial effect on *Candida* levels—the experimental group showed no increase in *Candida* levels as opposed to an increase in the control group. A larger study should be carried out using a sample size with enough colonized patients to better evaluate the effect of this product.

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DISCLOSURE

The fifth author, Bart De Wever, is employed by MSI Laboratories, the manufacturer of NitrAdine.

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