ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Differential Changes in Epithelial Thickness of Oral and Pathological Odontogenic Epithelia in Response to Inflammatory cell infiltrate

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Manuscript Info

Manuscript History:

Received: 15 September 2015
Final Accepted: 22 October 2015
Published Online: November 2015

Key words:

Inflammation, epithelial thickness, oral epithelium, dentigerous cyst, keratocystic odontogenic tumour

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Abstract

Background; Oral and odontogenic epithelia do not always fulfill the known histological features to be diagnosed as specific entities due to many factors including infection.

Objectives: This study investigated the changes in thickness of buccal epithelium, the epithelium of dentigerous cysts and the epithelium of keratocystic odontogenic tumours in relation to varying degrees of infiltration by inflammatory cells.

Methods: Histological preparations of biopsies of the buccal mucosa from 22 volunteers were compared with those from 23 dentigerous cysts and 18 odontogenic keratocysts for inflammatory cell infiltrate and epithelial thickness.

Results: Epithelia in dentigerous cysts showed an increase in thickness from a mean of 64.6 ± 8.2 µm to 178.2 ± 11.3 µm in tissues presenting with low and severe inflammatory cell infiltrate respectively. Likewise, epithelial thickness in keratocystic odontogenic tumours showed an increase from a mean of 69.3 ± 9.9 µm to a mean of 148.3 ± 11.3 µm in tissues presenting with low and severe inflammatory cell infiltrate respectively. In both cases, the change in epithelial thickness correlated with the degree of inflammatory cell infiltrate.

Conclusions: Inflammatory cell infiltration induces increased thickness of pathological odontogenic epithelia and this could present challenges to histological diagnosis of these conditions.

INTRODUCTION

The oral cavity has different types of mucosa that have specific features which distinguish them from other mucosa around the body. With regard to function, the oral mucosa is classified into three, namely lining, masticatory and specialized mucosa accounting for 60%, 25% and 15% of the total surface of the oral cavity respectively (Presland and Dale 2000). The oral mucosa may also be classified based on keratinization (orthokeratinized, parakeratinized or non-keratinized). Like most other mucosa, the structure of oral mucosa includes an overlying oral epithelium, a lamina propria and the submucosa.

The oral epithelium is organized in stratified layers called basal, spinous and superficial layers (Presland and Dale 2000). The structure and thickness of this epithelium can be affected by many factors including infective processes, immune conditions, trauma, nutritional deficiencies and even treatment modalities such as chemotherapy (Wardill,

Logan et al. 2015). Normal oral epithelium has unique histological features that enable oral pathologists to readily distinguish it from pathological entities arising from it or from other oral and facial tissues.

Keratocystic odontogenic tumour is defined as a benign unicystic or multicystic tumor of odontogenic origin with a characteristic lining of parakeratinized stratified squamous epithelium, with potential for aggressive and infiltrative behavior (Nayak, Singh et al. 2013). The tumour was previously classified as a cyst known as odontogenic keratocyst, but was reclassified as a tumour after it was noted to have aggressive biological potential (Eversole, Sabes et al. 1975). Keratocystic odontogenic tumours normally have a thin epithelium of about 5 to 7 cells in thickness (Telles, Castro et al. 2013)

Dentigerous cysts are developmental cysts of the jaws associated with the crown of an unerupted tooth. They are the most common developmental odontogenic cysts and often remain completely asymptomatic unless when infected or could be discovered on routine radiographic examination. Most dentigerous cysts are lined by a thin 2 to 3 cell layer thick cuboidal epithelium that is derived from reduced enamel epithelium (Benn and Altini 1996). Like oral epithelia, these pathological odontogenic epithelia do have unique histological features that enable oral pathologists to readily distinguish them from other pathological entities and from other oral and facial tissues.

In many cases, cysts of odontogenic origin do not fulfill the known histological features to be diagnosed as specific types of cysts. One of the most common causes of this is changes to their lining epithelium induced by inflammation (Ghogre and Singh 2014). Many oral pathologists report these cysts as 'infected dental cyst' or 'inflammatory odontogenic cyst'. This histological diagnosis is often considered with an element of doubt by clinicians who may not understand the limitations of histological diagnosis. There is therefore a need for a deeper understanding of the potential oral and odontogenic epithelial changes induced by various inflammatory processes. This study investigated the changes in thickness of oral (buccal) epithelium, the epithelium of dentigerous cysts and the epithelium of keratocystic odontogenic tumours in relation to varying degrees of infiltration by inflammatory cells.

MATERIALS AND METHODS

Study samples

Two groups of samples were used in this study. The first group consisted of samples collected from study participants for research purposes. The participants were clinically healthy adult volunteers undergoing surgical removal of wisdom teeth. They were requested to participate in the study by allowing a small piece of buccal mucosal tissue to be biopsied from the incision line during surgical removal of impacted wisdom teeth. The use of human subjects and tissues for research purposes was reviewed and approved by the regional Institutional Research and Ethics Committee (IREC) and all participants were informed that their tissues would be used for the purpose of research and were requested to sign consent forms. A total of 22 samples were collected in this group.

The second group consisted of samples previously diagnosed as either dentigerous cyst or keratocystic odontogenic tumour. These were selected from the laboratory archives and were included in the study only for comparison with those from buccal epithelium. There were a total of 23 samples of dentigerous cyst and a total of 18 samples of keratocystic odontogenic tumour.

Tissue preparation and staining

Tissue samples were fixed at room temperature in 4% buffered formalin. Biopsy specimens were grossed and taken through routine procedures for formalin fixed paraffin embedded preparations. Briefly, the formalin fixed tissues was washed in more formalin solution to remove any lose debris and then dehydrated through a series of graded alcohol (70%, 90%, 100%, 100% and100% ethanol 1 h each), and then through three changes of xylene for 1 h each. The tissues were then incubated overnight at 65°C in Paraplast Plus paraffin (Thermo Fisher Scientific) before embedding. The blocks were then stored at room temperature to await sectioning.

The tissue blocks were sectioned at 5 μm thickness and placed on glass slides. The slides were dewaxed by placing them in a slide holder and taking them through xylene for 20 min with gentle agitation. The slides were then rehydrated by placing them in graded alcohol (100%, 100%, 90%, and 70% ethanol for 3 min each) and then gently in running tap water. The tissues were stained by placing them in Harris haematoxylin (Sigma Aldrich, Darmstadt, Germany) for 10 minutes. They were then washed slowly in running water, and gently distained (using 1% HC1 in

95% ethanol), rinsed again in running tap water for 5 min, then placed in Scott's tap water (bluing solution) (Leica Biosystems GmbH, Wetzlar, Germany) for 3 min, then stained in eosin (Sigma Aldrich, Darmstadt, Germany) for 2 min. The tissues were then dehydraded in graded elcohol (70%, 90%, 100%, 100% and100% ethanol 5 h each) then cleared in three changes of xylene for 5 min each and mounted using Permount mounting medium (Fisher Scientific, Fair Lawn, NJ, USA). Upon drying, the hematoxylin-eosin stained slides examined under light microscopy.

Histological assessment

Histomorphometric analysis of tissue sections was conducted using a computer based digital image analysis software (DinoCapture 2.0) using the DinoEye AM4023 digital eyepiece camera (AnMo Electronics Corporation, Taipei, Taiwan) connected to a computer via USB. Analysis of all tissue sections was done at similar settings and magnification under an Olympus CX31 light microscope (Olympus Corporation, Tokyo, Japan). The thickness of the epithelium was determined by drawing arbitrary lines running vertically from the surface to the basement membrane as previously described (Lukandu, Koech et al. 2015) and reading off the length/depth as was indicated by the software. The tissues were all assessed for epithelial thickness and compared with other samples.

To assess degree of inflammation, photographs of random sections from different locations within the underlying connective tissue measuring 100 μm^2 were taken. All chronic and acute inflammatory cells from selected sections in the digital photographs were identified and counted based on their size, shape, and the intensity of staining. Only cells that were clearly identifiable as lymphocytes, polymorphonuclear leukocytes and plasma cells were included in the count. This was done at X400 magnification and the final value for each tissue sample was deduced from an average of three separate counts. Samples with inflammatory cell values below 30 cells per 100 μm^2 were classified as low or no inflammation, those with 31 to 80 cells per 100 μm^2 were classified as medium or moderately inflamed and those with over 80 cells 100 μm^2 were classified as severely inflamed.

Statistical analysis

Data analysis and generation of figures was done using Sigma Plot software version 12.5 (Systat Software, Inc., San Jose, CA, USA). Data sets from the groups were first subjected to Shapiro-Wilk normality test and then compared using one way analysis of variance (ANOVA) or Kruskal Wallis Test. This was followed by multiple comparisons using the Holm-Sidak method to determine the levels of significance between specific groups. *p*-values less than 0.05 were considered significant. Data were presented as means ± standard error of the means.

RESULTS

Whereas the buccal mucosa did not show much change in the thickness of its epithelium (Figure 1), the epithelium in dentigerous cysts (Figure 2) and the epithelium in keratocystic odontogenic tumours (Figure 3) showed increased thickness with increase in degree of inflammatory cell infiltrate. Samples from the buccal mucosa presenting with low inflammatory cell infiltrate had a mean epithelial thickness of 324.6 ± 13.7 µm, and this reduced only slightly to 302.4 ± 16.8 µm in those from tissues with severe inflammatory cell infiltrate. This difference was however not statistically significant.

On the contrary, epithelia in dentigerous cysts showed a significant increase in thickness from a mean of 64.6 ± 8.2 µm in tissues presenting with low inflammatory cell infiltrate to a mean of 178.2 ± 11.3 µm in tissues presenting with severe inflammatory cell infiltrate (p = 0.016). Likewise, epithelial thickness in keratocystic odontogenic tumours showed a significant increase from a mean of 69.3 ± 9.9 µm in tissues presenting with low inflammatory cell infiltrate to a mean of 148.3 ± 11.3 µm in tissues with severe inflammatory cell infiltrate (p = 0.004) (Table 1).

Further analysis of tissues from selected dentigerous cysts showed a positive correlation between epithelial thickness and degree of inflammation (n = 8, r = -0.805, p = 0.016) (Figure 5). A similar finding was also observed upon further analysis of epithelia from selected keratocystic odontogenic tumours (n = 8, r = -0.878, p = 0.004) (Figure 6). This correlation was not observed among tissues drawn from the buccal epithelium (Figure 4).

Table 1. Variation of epithelial thickness in oral and odontogenic epithelia with degree of inflammatory cell infiltrate.

Degree of inflammation	Number of samples	Inflammatory cells per 100 μm^2	Mean thickness of epithelium (µm)

			(mean)	
Buccal epithelium		22	-	-
	Mild	7	13	324.6 ± 13.7
	Moderate	8	63	311.0 ± 19.6
	Severe	7	116	302.4 ± 16.8
Dentigerous cysts		23	-	-
	Mild	8	7	64.6 ± 8.2
	Moderate	6	43	136.0 ± 18.9
	Severe	9	108	178.2 ± 11.3
Keratocystic odontogenic tumour		18	-	-
	Mild	7	6	69.3 ± 9.9
	Moderate	5	56	125.0 ± 13.1
	Severe	6	98	148.3 ± 11.3

Figure 1. Histological presentation of tissue samples from buccal oral epithelium. A) low inflammatory cell infiltrate, B) moderate inflammatory cell infiltrate and C) severe inflammatory cell infiltrate.

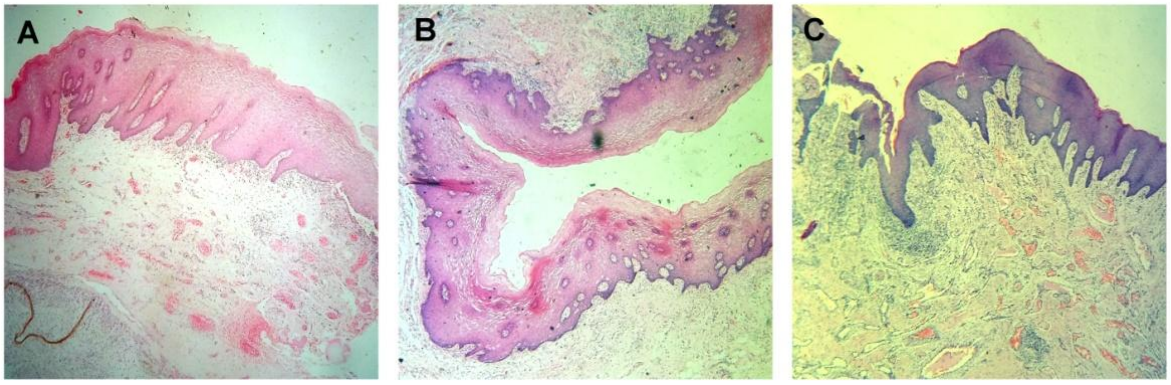


Figure 2. Histological presentation of tissue samples from dentigerous cysts. A) low inflammatory cell infiltrate, B) moderate inflammatory cell infiltrate and C) severe inflammatory cell infiltrate. Notice the anastomosing arcades of the epithelium in panel C.

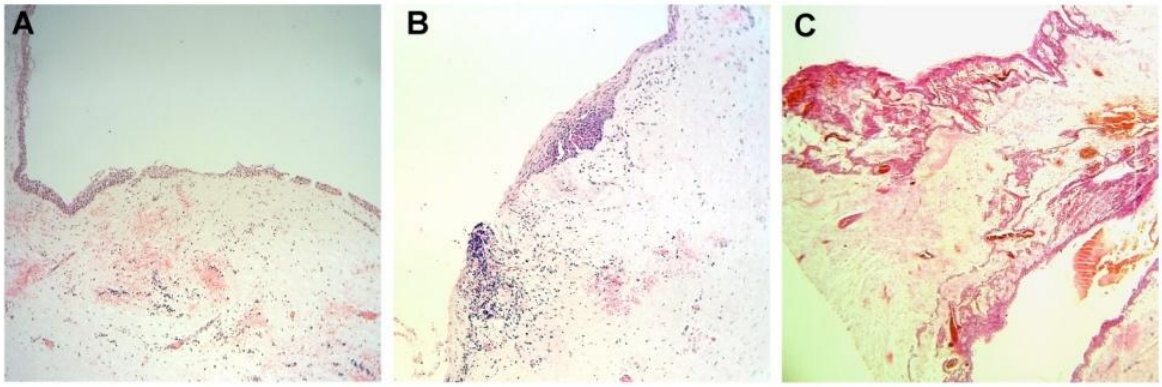


Figure 3. Histological presentation of tissue samples from keratocystic odontogenic tumours. A) low inflammatory cell infiltrate, B) moderate inflammatory cell infiltrate and C) severe inflammatory cell infiltrate.

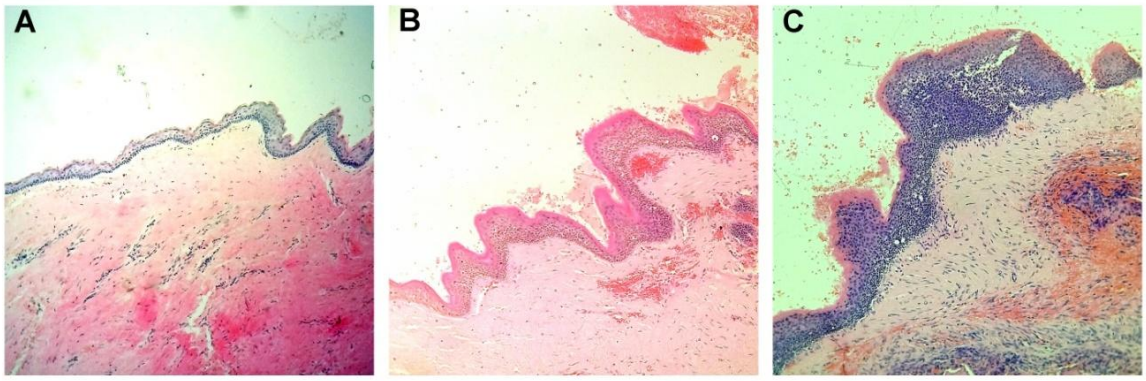


Figure 4. Analysis of thickness of buccal oral epithelium in tissues with varying degrees inflammatory cell infiltrate.

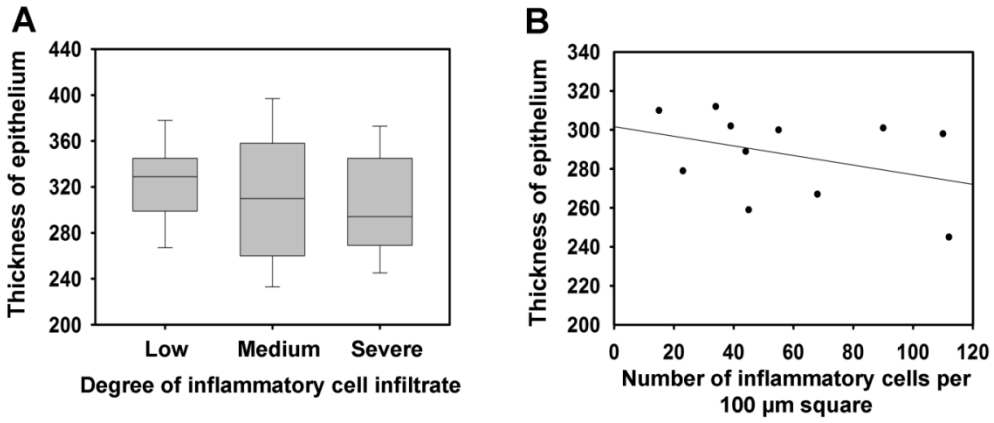


Figure 5. Analysis of thickness of epithelium in dentigerous cysts with varying degrees inflammatory cell infiltrate.

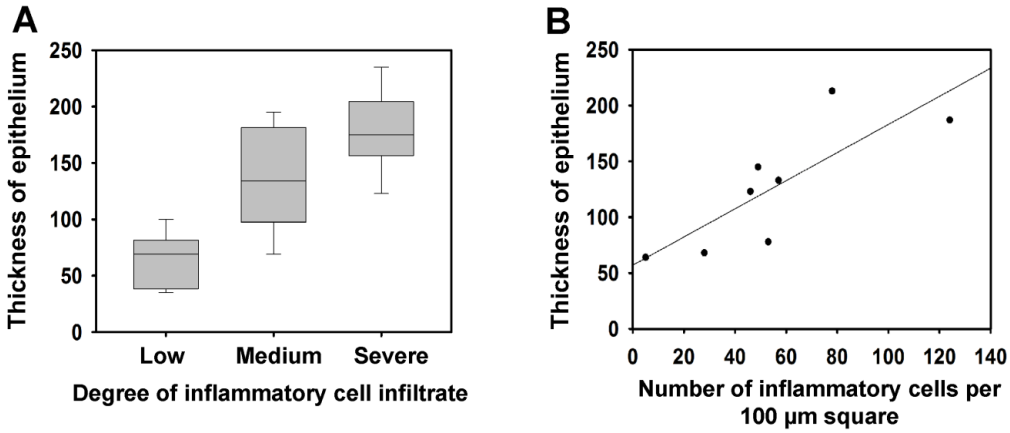
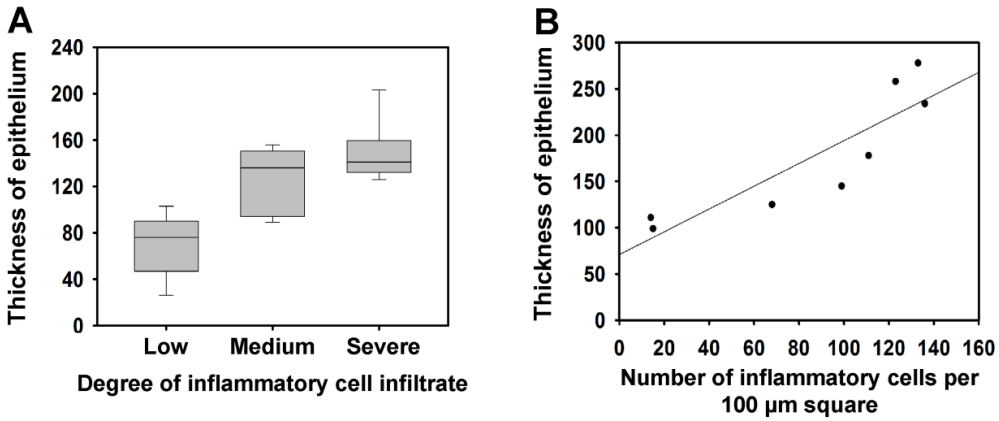


Figure 6. Analysis of thickness of epithelium in keratocystic odontogenic tumours with varying degrees inflammatory cell infiltrate.



DISCUSSION

This study compared changes in epithelial thickness and their relation to the degree of inflammatory cell infiltrate in tissue samples of the buccal mucosa, dentigerous cysts and keratocystic odontogenic tumours. The study demonstrated a profound change in epithelial thickness to be among the changes that occur in odontogenic epithelium upon infective inflammation. From the study findings, this change could be up to a three or four fold increase in epithelial thickness. The study also demonstrated that the change in epithelial thickness is depended upon the degree of infiltration by both acute and chronic inflammatory cells.

Interestingly, this change was not observed in oral (buccal) epithelium upon infiltration by inflammatory cells into the underlying connective tissue. Normal thickness of epithelium of the oral mucosa varies depending on the site in the oral cavity. The thickest epithelium is found in the buccal mucosa where it measures approximately 290 µm (Prestin, Rothschild et al. 2012). In this study, mean epithelial thickness in the buccal mucosa was found to be 324 µm and was slightly reduced to 324 µm among tissues depicting severe inflammatory cell infiltrate. Even though this effect was not significant, data from this study appeared to suggest that, in response to infiltration by chronic inflammatory cells, the oral epithelium could show reduced thickness and even show signs of atrophy. Further studies are needed to confirm these findings and to further explore the differential changes in oral and odontogenic epithelia in response to chronic inflammation.

Keratocystic odontogenic tumours normally have an epithelium of about 5 to 7 cell thickness (Telles, Castro et al. 2013) while majority of dentigerous cysts are lined with a very thin 2 to 3 cell layer thick cuboidal epithelium (Benn and Altini 1996, Yeo, Rosnah et al. 2007). Upon inflammation, such as seen following marsupialization of keratocystic odontogenic tumours, the lining appears to become thicker and also appears to change and resembles that of the normal oral mucosa (Brondum and Jensen 1991, Marker, Brondum et al. 1996). When enclosed within bone, infected cysts could show marked and hyperplastic epithelium that often exhibit deep rete ridges and even anastomosing arcades of epithelium mimicking radicular cysts (Benn and Altini 1996).

According to previous studies, infiltration by inflammatory cells was identified as a factor contributing to the change in epithelial thickness of these tumours to resemble that of normal oral mucosa or radicular cysts. In one such study, these keratocystic odontogenic tumours were shown to lose their epithelial keratinization and to resemble the epithelium known to occur in radicular cysts (Stoelinga 2001). In other studies, it was shown that infected keratocystic odontogenic tumours depicted increased proliferative activity in the epithelial cells disrupting their typical epithelial structure (de Paula, Carvalhais et al. 2000, August, Faquin et al. 2003). Findings in this study therefore agree with previous observations.

One previous study compared various histologic features of gingival mucosa among smokers and non smokers and showed that an increase in epithelial thickness occurred in response to tobacco use, but appeared to be independent of the presence of periodontal inflammation (Prakash, Rath et al. 2014). Unlike odontogenic epithelium, the oral epithelium could therefore be less susceptible to increased thickness in response to infective inflammatory processes. An alternative explanation could be that the oral epithelium, especially at its thickest within the buccal mucosa, exists at an optimal and physiological thickness where further increase in thickness is difficult unless the surface epithelium becomes hyperkeratinized and nonvital. This view could be supported by a common observation that stressful forces on the oral epithelium that precipitate increased epithelial thickness almost always also present with hyperkeratinization.

CONCLUSION

This study found a positive correlation between epithelial thickness and degree of chronic inflammatory cell infiltrate within the underlying connective tissue of dentigerous cysts and keratocystic odontogenic tumours. Of clinical and diagnostic importance is that these changes in epithelial thickness do present challenges to histological diagnosis of these conditions. Further studies are recommended to understand the processes leading to these changes, and to suggest new methods of identifying these lesions even in their changed state.

ACKNOWLEDGEMENT

I wish to thank the staff at the Moi University School of Dentistry for their assistance in clinical procedures and the histopathology laboratory staff at Moi Teaching and Referral Hospital for their technical support.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest regarding the publication of this paper.

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