Original Article

Evaluation of Efficacy of Foldscope – A Paper Microscope to be Used as a Chairside Diagnostic Tool in Oral Dysplastic Lesions: A Comparative Study

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Abstract

Introduction: Precancerous lesion of the oral mucosa consists of a group of diseases which sometimes resemble each other leaving the clinician in a diagnostic dilemma. Etiology of these diseases varies geographically with most frequently being tobacco use, alcohol drinking, chewing of betel quid containing areca nut, and solar rays. The long-standing practice of these lifestyle habits causes an alteration in the mucosal barrier level leading to malignant transformation. Earlier, the diagnosis of malignant transformation was confirmed using biopsy, but the advent of exfoliative cytology showed that histological features of a cell undergoing transformation are distinctive during early stages. Early diagnosis can be lifesaving, along with chairside adjunct tools that can facilitate the clinician for better diagnosis and use it as an explanatory tool for patients. **Aim:** The aim of this study was to test the efficacy of foldscope as a chairside diagnostic tool to detect dysplastic changes in potentially malignant lesions affecting the oral cavity. **Materials and Methods:** This was a cross-sectional comparative study of a total of 54 individuals clinically diagnosed with oral premalignant lesions. Exfoliative cytological smears were taken and observed under light microscope and foldscope. After Papanicolaou stain, it was subjected to cytomorphometric analysis. **Results:** Cytological changes in potentially malignant lesions detected using foldscope were appreciable and found to be a mirror image of the routine light microscope. **Conclusion:** Morphological parameters assessed by foldscope proved to be employed in routine practice as well as in the mass screening of oral lesions.

Keywords: *Foldscope, light microscope, Papanicolaou stain, potentially malignant oral lesions*

Introduction

Early diagnosis of potentially malignant or dysplastic lesions plays a pivotal role in instigating the significance of biopsy and also in formulating treatment modalities for patients.[1] This, in turn, has a direct impact on both the quality and quantity of the patient's life. Clinicians play an important role in early detection of these lesions, as most patients who visit dental hospitals with other dental disorders are not treated for the associated red or white lesions.

Although biopsy is the gold standard procedure for the diagnosis of dysplastic lesions, from a patient's point of view, it is still a nightmare.[2] Generally dental practitioners around the world do not perform biopsy in their clinical practice due to lack of expertise.[3] Other noninvasive diagnostic tools like Vizilite have been stated to be clinically innocuous and ineffective in detecting oral dysplastic lesions.[4]

Few have stated that exfoliative cytology seems to be less technique sensitive,[1] but again, it involves transportation of smear to a laboratory as most clinicians do not have a light microscope in a clinical setup.

Hence, developing a cost-effective chairside tool that should be efficient and less technique sensitive is of prime importance in screening and detecting early dysplastic lesions. Foldscope is one such device, which is nothing but a paper microscope that is a versatile, sturdy, reliable, cost-effective, lightweight device with multiple lenses that can provide magnification from ×140 to ×2000.[5] Foldscope is similar to that of a light microscope, which uses visible light and a system of lenses to magnify images of small objects.[6] Compared to light microscope, cost, maintenance and the technique involved is not an issue in the

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case of foldscope. However, its use in diagnostic pathology is yet to be proved. Hence, in this study, we are trying to test the efficiency of a cost-effective device, the foldscope in diagnosing and grading oral dysplastic lesions.

Aim

This study aims to test the efficacy of a cost-effective, efficient, and less technique-sensitive device called foldscope, to be used as a chairside diagnostic tool to detect dysplastic changes in potentially malignant lesions affecting the oral cavity in a simple dental setup.

Objectives

Primary objective

- To evaluate the efficacy of foldscope to be used as a chairside diagnostic tool
- To compare foldscope and light microscope in precise diagnosis of exfoliative cytology smears of oral dysplastic lesions – by grading of smears
- Analysis of the photomicrographic images obtained through both foldscope and light microscope in × 400 for cytomorphometric changes through Java imaging processing program - ImageJ software version 1.47.

Secondary objective

- To assess the efficiency of foldscope in combination with image analysis using ImageJ software detecting cytomorphometric changes
- To compare the diagnosis obtained by means of grading by observers and by image analysis by software.

Exploratory objective

- To compare the time depleted for diagnosis, both by A4 sheetand light microscope, with and without image analysis software.

Materials and Methods

Type of study

This was a cross-sectional comparative study and product testing.

Study population

Patients with oral premalignant lesions, visiting the department of oral medicine and radiology, were included in the study.

Sample size

A total of 54 individuals with suspicious oral premalignant lesions were recruited for the study [Figure 1].

Sample size calculation

It was done using G*Power software:

Input:	Effect size f	=	0.5000000
	α err prob	=	0.05
	Power (1−β err prob)	=	0.95
Number of groups		=	2.

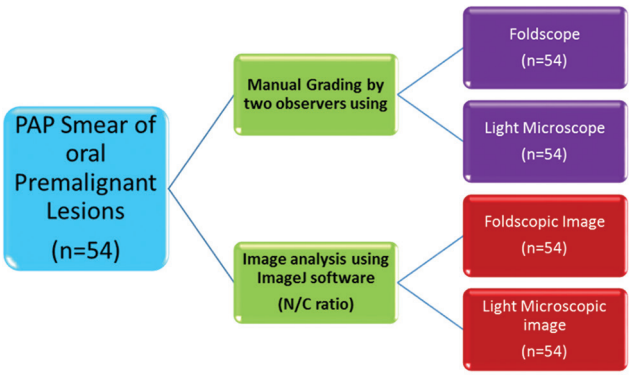

Figure 1: Analysis of sample

Output: Noncentrality parameter λ	=	13.5000000
Critical F	=	4.0266314
Numerator df	=	1
Denominator df	=	52
Total sample size	=	54
Actual power	=	0.9500773.

Selection criteria

Inclusion criteria

- Age – 25–50 years [Figures 2 and 3]
- Gender – Male and female
- Patients clinically diagnosed with oral premalignant lesions.

Exclusion criteria

- Patients with other associated systemic illnesses
- Patients diagnosed with other mucocutaneous disorders
- Patients diagnosed with allergic lesions
- Patients who have undergone biopsy
- Patients who were previously diagnosed with oral malignancy
- Patients who have undergone chemotherapy or radiation therapy.

Equipment used

- Cytobrush (Cytobrush Plus GT)
- Glass slides
- Spray Fixer (Cytology Fixative, Leica)
- Rapid Papanicolaou stain (PAP) (Biolab Diagnostics, Boisar)
- Foldscope version 2.0
- Light microscope (Lawrence and Mayo LM-52-6000)
- ImageJ software version 1.47
- Digital camera – Resolution 1080 × 1920 pixels.

Data collection procedures and instruments used

- The study involved 54 patients clinically diagnosed with oral premalignant lesions. The entire protocol procedures were explained at the beginning of the study to each patient, and written informed consent was obtained

- Consecutively, cytological smears were made from the lesional area using a Cytobrush Plus GT. The head of the Cytobrush cell collector was moistened with water and firmly held against the mucosa of the lesional area. Then, gentle pressure was applied to the brush until the bristles curled or tiny bleeding spots were evident. Further, the brush was rolled over the lesional site and rotated for 10 full turns. The Cytobrush cell collector was then rolled on glass slides by applying a continuous motion from one end of the slide to the other
- The resulting smears were fixed with Spray fixative (Cytology Fixative, Leica)
- The spray-fixed smears were stained by a commercially available Rapid PAP stain kit (Biolab Diagnostics, Boisar, Maharashtra, India).

Procedure

- Hydration: 3–5 min s wash in tap water
- Nuclear staining: Nuclear stain for 60 s and then washed in tap water
- Bluing: 3–5 drops of Scott's solution for 20 s and washed. Blotted out the excess
- Dehydration: Dehydrating solution for 60 s
- Cytoplasm staining: Dip the slide in Coplin jar for 60 s
- Washing: Washed in tap water and blot the excess
- Dehydration: Dehydrating solution – 60 s – let dry
- Xylene: Rinse in xylene
- Mounting: Mount with coverslip using a drop of digital picture exchange.
- The entire staining procedure took about 4–5 min
- The smears were double blinded before analysis
- Assembly of foldscope – The foldscope kit comprised foldscope paper components, ball lens, button cell battery, surface-mounted light-emitting diode (LED), switch, copper tape, and polymeric filters. Computer aided designing. The assembly of the kit was done following the instructions given in the manual [Figure 4] [1].
- Following which the smears were observed by two observers individually through foldscope, light microscope and the smears were graded based on the parameters mentioned below.

Grading of exfoliated smears

- Class I (normal): It indicates that only normal cells were observed [Figure 5]
- Class II (atypical): It indicates the presence of minor atypia but no evidence of malignant changes
- Class III (indeterminate): This is an in-between cytology that separates cancer from noncancer diagnosis. The cells display wider atypia that may be suggestive of cancer, but they are not clear-cut and may represent

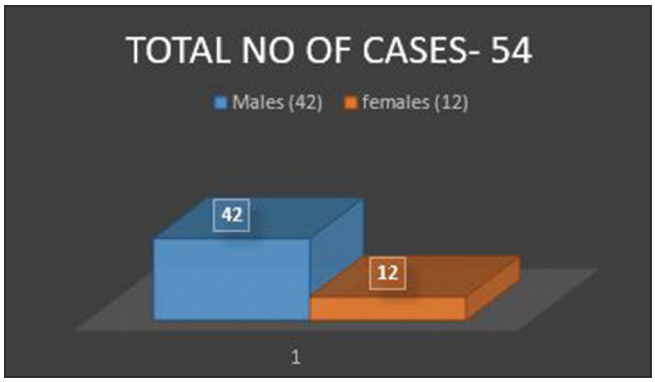

Figure 2: Male and Female patient distribution

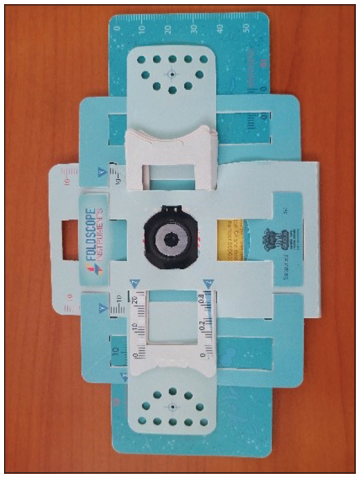

Figure 4: Fully assembled foldscope

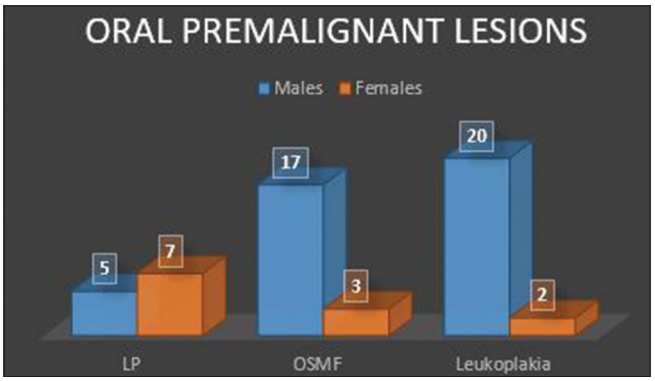

Figure 3: Oral Premalignant Lesions seen in the patients (LP-Lichen Planus, OSMF- Oral submucous Fibrosis)

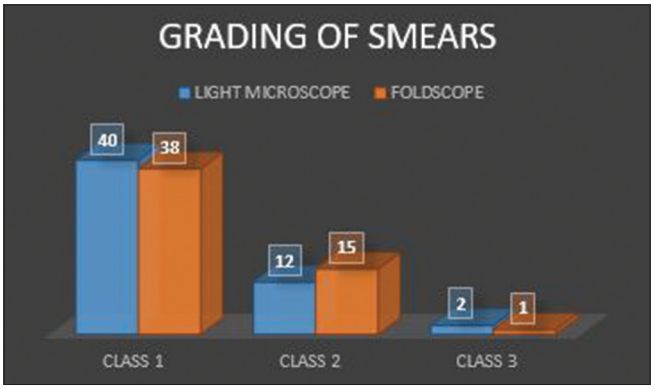

Figure 5: Grading of smears using Light Microscopic and Foldscope (Manual)

precancerous lesions or carcinoma *in situ*. Biopsy is recommended
- Class IV (suggestive of cancer): A few cells with malignant characteristics or many cells with borderline characteristics. Biopsy is mandatory
- Class V (Positive for cancer): Cells that are obviously malignant. Biopsy is mandatory[2]
  - Photographic images of smears were obtained through foldscope and conventional light microscope [Figures 6 and 7]
  - Images were taken using both foldscope and light microscope under ×400 magnification and were subjected to cytomorphometric study through ImageJ software version 1.47 [Figure 8].

Image analysis procedure

Fifty cells per individual, which were unfolded with clear outline was selected for the study. Cells were analyzed for cellular area, and nuclear area, with which nuclear-cytoplasmic (N/C) ratio was calculated using the ImageJ software. The sampling was done in a stepwise manner, moving the slide from the left upper corner to the right and then down in order to avoid measuring the same cells again. The measurements were obtained in terms of square pixels.[3]

- All the recorded data were subjected to statistical analysis for testing null and alternate hypothesis:
  I. Null hypothesis (H_0) – There is no significant difference in efficiency of light microscope and foldscope [Figure 9]
  II. Alternate hypothesis (H_1) – There is a significant difference in the efficiency of light microscope and foldscope.

Results

The study was conducted to evaluate the efficiency of foldscope to be used as a chairside diagnostic tool, in detecting cytological changes in potentially malignant oral lesions, compared to that of routine light microscope.

A total of 54 patients were recruited for the study [Figure 10]. The data obtained by manual and software analysis were evaluated statistically. The data were subjected to one-way analysis of variance, done using IBM Statistical Package for the Social Sciences (SPSS) software.

Discussion

Invasive oral squamous cell carcinoma is often preceded by the presence of clinically identifiable premalignant changes in the oral mucosa.[1] Gaurav Sharma *et al*. had stated that timely recognition of oral cancer and its various predecessors continued to be the most excellent strategy to safeguard the survival rates of patients and better life quality of patients.

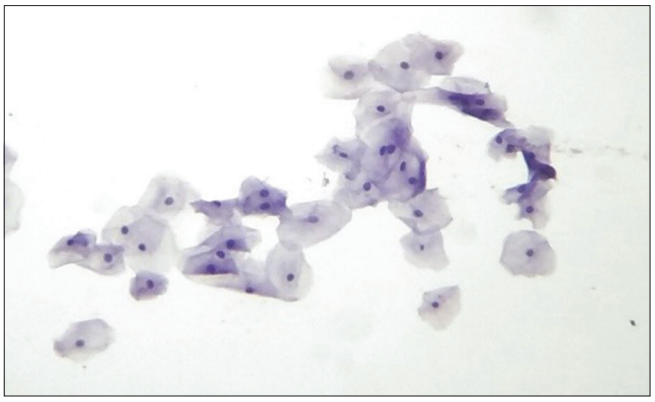

Figure 6: PAP stained slide – Image obtained through Light microscope

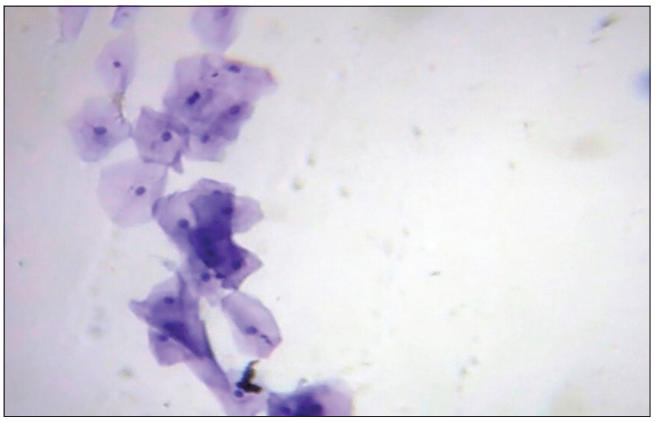

Figure 7: PAP stained slide – Image obtained through Foldscope

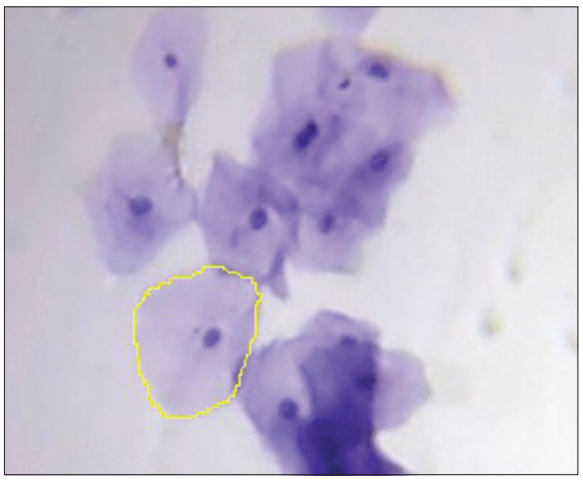

Figure 8: Tracing of cell outline using ImageJ software

Early detection of oral premalignant lesions is the key way to lessen the morbidity and mortality rates and also enable the clinician to intervene the progress of the lesion at an early stage.[7] The conventional oral examination has various disadvantages like false-positive findings, including psychological trauma, overdiagnosis, increased human and financial resources, and recognition of varied clinical presentations of the premalignant lesion.[8]

Various studies have demonstrated the use of different equipment and modalities to detect the condition at an earlier stage for diagnostic accuracy. This includes usage of VELscope, vital tissue staining, tissue fluorescence spectroscopy, chemiluminescence, and confocal *in vivo* microscopy.[4]

The normal epithelial lining is generally prone to regular exfoliation, and the study of theses exfoliated cells is termed as exfoliative cytology. In general, any benign or recurrent malignant condition leads to loss of cell adhesion and causing exfoliation of cells. These cells can be analyzed both quantitatively and qualitatively.[9] The cytomorphometric analysis of these cells has escalated the role of these exfoliated cells in diagnostic pathology.[5]

In contrast, few authors debate the efficiency of exfoliated cells in identifying potentially malignant lesions. However, others state that cytological smears are useful in diagnosing the alterations seen in long-standing lesions such as leukoplakia and oral submucous fibrosis. Previous studies performed on oral exfoliative cytology have concluded that the technique is useful in premalignant lesions.[6]

Various awareness studies have claimed that dentists were aware of the recent advances in the field of cytology, but as known exfoliative cytology does have its limitations, the procedure requires the necessary armamentarium, a microscope, and above all, a qualified oral pathologist to interpret the smears.[10] Researches on the techniques which are simpler, noninvasive, economically feasible, and less time-consuming and those which require minimal armamentarium at the site of the collection and a method to make chairside diagnosis possible would inculcate the possibility of routine cytodiagnosis.[7]

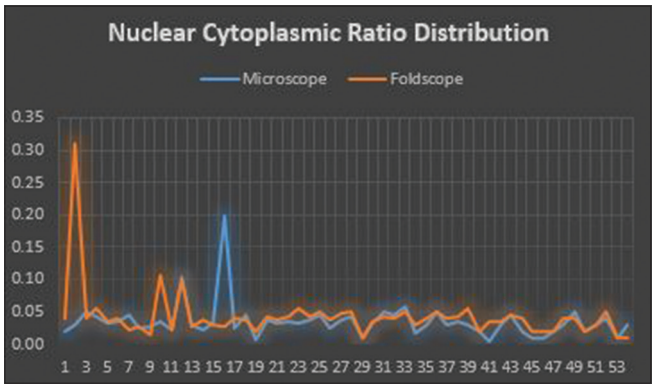

Figure 9: Nuclear Cytoplasmic ratio of images, evaluated using ImageJ software

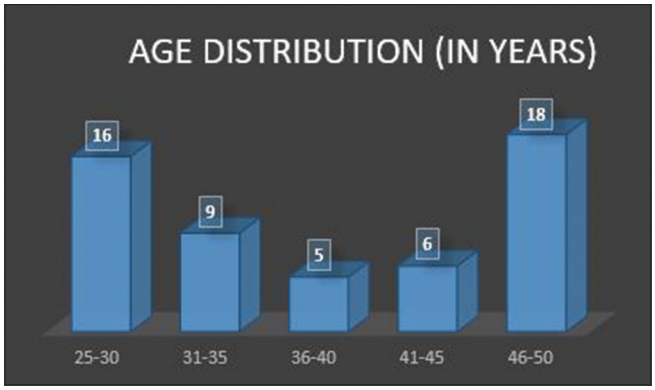

Figure 10: Age distribution of the patients

This study aimed at exploring the cytomorphometric analytical method using a chairside tool-foldscope. Our study was first of its kind, which was carried out to assess the cytomorphometric features of cells obtained from buccal scrapings in some of the most common oral potentially malignant disorders using foldscope.[11] Till date, very few studies have been conducted using foldscope. Walliulah *et al*.[12] in their study have used foldscope to compare the morphology of nonhuman histopathological samples.[13] The study showed that the morphological image so obtained from normal microscope and foldscope of nonhuman histopathological samples were similar.[14] Our study also replicated similar kind of results; as shown in Table 1, the grading of smear did not show a significant difference ($P > 0.05$); as in Table 2, it shows that 40 and 38 cases were diagnosed as Class I smear by light microscopic and foldscopic examinations, respectively; 12 and 15 cases were diagnosed as Class II smear by light microscopic and foldscopic examinations, respectively; and 2 and 1 cases were diagnosed as Class III smear by light microscopic and foldscopic examinations, respectively, which depicted only a minor variation which was statistically not significant. Second, the image analysis done to calculate N/C ratio with images captured using light microscope and foldscope [Table 3] presented with no significant difference ($P > 0.05$) between the images obtained from microscope and foldscope, with the mean value of N/C ratio being 0.035 for light microscope and 0.043 for foldscope, respectively. These findings indicate that foldscope can be used as an effective tool in mass

Table 1: Manual grading of smears - analysis of variance

	Examination	*n*	*F*	Significant, *P*
Grading of smears	Light microscope	54	0.034	0.854, >0.05 (not significant)

The statistical analysis of N/C ratio, obtained by both light microscope and foldscope, demonstrated no significant difference P value – 0.241(P>0.05)

Table 2: Grading given using light microscope and foldscope

Grades	Light microscope	Foldscope
Class I	40	38
Class II	12	15
Class III	2	1
Class IV	0	0
Class V	0	0
Total	54	54

Table 3: Statistical analysis of nuclear-cytoplasmic ratio obtained using ImageJ software

Cytological parameter	Microscope	*n*	Mean	SD	95% CI for mean		*F*	Significant, *P*
					Lower bound	Upper bound		
N/C ratio	Light microscope	54	0.035	0.027	0.027	0.043	1.392	0.241, >0.05 (not significant)
	Foldscope	54	0.043	0.041	0.032	0.054		

N/C ratio: Nuclear cytoplasmic ratio, SD: Standard deviation, CI: Confidence interval

screening and as a routine chairside diagnostic tool to rule out dysplasia in high-risk patients. The results were concurrent with that of the study conducted by Bruno M *et al*.[15] in cervical cancer patients, wherein the comparison of images between optical microscope and foldscope presented with similar cytological features.[16] The study demonstrated decrease in time consumed for transportation of the slide for microscopic examination by using a chair side diagnostic tool However, during the course of study, few drawbacks were also evident; it includes low contrast and blurring of periphery of the image taken through foldscope. Otherwise, foldscope proves to be an effective chairside diagnostic tool in assessing alterations in oral exfoliated cells.

Conclusion

Although awareness about exfoliative cytology as a preliminary diagnostic tool is explicit among dentists, the ability to implement cytology as a part of routine practice is very less.[17] Various reasons have been quoted for the same, which includes the armamentarium required, especially the microscope, without which diagnosis is impossible. This lacuna is the key for our research, by using a simple cost-effective paper microscope – foldscope instead of a compound microscope as a chair side diagnostic tool. Excluding the minor errors, the usage of foldscope attached with that of a microscope proves to be an effective diagnostic tool in detecting alterations in oral exfoliated cells.

Summary

This study mainly focused on assessing the efficiency of foldscope, a paper microscope to be used as chairside diagnostic tool. The smears taken from oral premalignant lesions were subjected to Rapid PAP staining, followed by which the slides were observed in light microscope and foldscope, following which the cells were graded. Simultaneously, images were captured through both light microscope and foldscope. The images obtained were assessed using ImageJ software. Both the manual and digital evaluation showed that the morphological parameters assessed by foldscope more or less remained the same as seen in light microscope, proving the ability of foldscope to be employed in routine practice as well as in mass screening of oral lesions.

Suggestions

Based on our study, we have concluded that further studies are to be framed to develop an inbuilt LED foldscope and a lens with more precise resolution.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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