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INCIDENCE OF CANDIDA INFECTION IN ORAL CANCER PATIENTS PRE AND POST RADIOTHERAPY TREATMENT AT A TERTIARY CARE CENTRE IN KANPUR

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ABSTRACT

Aim and Objective: To find out incidence of Candida infection in oral pre and post cancer patients and to find out most effective antifungal agent for empirical treatment.

Material and methods: A total of 60 patients have been selected for this study, who have been diagnosed for oral cancer from January to June 2016 at Rama Medical College Hospital and Research Centre, Kanpur. All isolates were further speculated and tested for antifungal susceptibility according to CLSI guidelines 2016.

Results: Out of 60 oral cancer patients, 30 samples were collected from patients before radio therapy and 30 samples were collected from patients after radio therapy. Out of 30 samples from pretreatment cases, 14 were found positive for Candida species and from post treatment cases, 19 were observed positive for *Candida* species. Out of 14 Candida isolates from pretreatment cases, 6 were linked to *C. albicans*, 3 were connected to *C. dubliniensis*, 3 were concerned to *C. krusei* and 2 were belonged to *C. parapsilosis*. Out of 19 Candida isolates from post treatment, 7 were linked to *C. dubliniensis*, 4 were concerned to *C. albicans*, 4 were connected to *C. krusei* and 4 were related to *C. glabrata*, among patients post treatment. Antifungal susceptibility test result showed Amphoterocin B was most sensitive drug for all *Candida* species except *C. parapsilosis* and Fluconazole was most resistant for all *Candida* species.

Conclusion: *Candida albicans* was predominant isolates in oral cancer patients and most effective antifungal drug was amphoterocin B and least effective antifungal drug was found fluconazole. This study has showed a lot of variation in drug sensitivity, and also has showed irrational use of antifungal drugs. Thus, fungal culture and anti fungal susceptibility tests are necessary for effective treatment. After radiotherapy Candida infection was recorded more common than that of pre radio therapy, it shows radio therapy increases the risk of local Candida infection due to immune-suppression.

KEYWORDS : Oral cancer, Candida, Antifungal agents, radiotherapy

INTRODUCTION

The Candida genus belongs to the phylum fungi imperfecti, the order moniliales and family Cryptococcaceae [1]. Twenty of these species are considered as significant pathogens causing various infections in human being and out of twenty the eleven are well known opportunistic pathogens i.e. *Candida albicans, C. tropicalis, C. krusei, C. glabrata, C. guilliermondii, C. parapsilosis, C. lusitaniae, C. kefyr, C. rugosa, C. dubliniensis, C. viswanathi* [2]. Oral candidiasis is a common fungal infection which affecting patients suffering from cancer.

Cancers are the most common cause of death in adults [3]. Oral cancer is any malignant neoplasm which is found on the lip, floor of the mouth, cheek lining, gingiva, palate or in the tongue. Oral cancer is among the top three types of cancers in India [4]. Severe alcoholism, use of tobacco like cigarettes, smokeless tobacco, betel nut chewing and human papilloma virus (HPV) are the most common risk factors for oral cancer [5, 6]. Oral cancer may also occur due to poor dental care and poor diet [7].The incidence of oral cancer is highest in India, south and south East Asian countries. In India, 90-95% of the oral cancers are squamous cell carcinoma [8]. The international agency for research on cancer has predicted that India's incidence of cancer will increase from 1 million in 2012 to more than 1.7 million in 2035. This indicates that the death rate because of cancer will also increase from 680000 to 1-2 million in the same period [9].

A case control study from India has been demonstrates that oral cancer is interrelated with low income. Low social economic class is interrelated with factors like nutrition, health care, living condition and risk behaviors which contribute to the development of oral cancer [10]. In many low-income and middle-income countries, including India, most of the population does not have access to a well organized and well regulated cancer care system. A diagnosis of cancer often leads to high personal health expenditures. Such expenditures can push entire families below the poverty line and may threaten social stability [11]. No significant advancement in the treatment of oral cancer has been found in recent years, though the present treatments improve the quality of life of oral cancer patients but the overall survival rate of 5 years has not improved in the past decades.

In India, 20 % (100000 persons) population is affected by oral cancer which accounts for about 30% of all types of cancer [12]. Over 5 people in India die every hour everyday because of oral cancer and the same number of people dies from cancer in oropharynx and hypo pharynx [13]. Cancer registration is not compulsory in India, so the true incidence and mortality may be higher, as many cases are unrecorded and loses follow up [14]. None of the national registry provides cancer incidence or mortality data for India. However, the National Cancer Registry Program provides population-based data from a selected network of 28 cancer registries located across the country [7]. A number of studies use data from urban and rural cancer registries established at the national regional level. Urban registries includes Delhi, Mumbai and Chennai and rural registries include Barshi, Dindigul, Manipuri, Karunaga-pally, Eranakulam, Srikakulam and Bhavnagar cancer is of significant public health importance to India [9]. Mostly it is diagnosed at later stages which result in low treatment outcomes and high costs. Many patients cannot afford such types of treatment. In rural areas, patients have inadequate access to trained providers with very limited health services. Hence the delay is largely associated with advanced stages of oral cancer. Earlier detection of oral cancer offers the best chance for long term survival and has the potential to improve treatment outcomes and make healthcare affordable [15]. Mostly, oral cancer affects the people from the lower socioeconomic status of society and people in rural area due to a higher exposure to risk factors such as the use of tobacco [16, 17].

According to the statistics, in 2012 the incidence of oral cancer in

India is 53842 in males and 23161 in females [11]. Oral cancer is considered to be a disease which occurs in elderly people. However, most of the oral cancer cases occur between the ages of 50 to 70 years, but it could also affect children as early as 10 years. Incidence of oral cancer increases by age [18]. The commonest age is the fifth decade of life [19]. Considering the gender in all the age groups, men are more affected than women. In India, men are two to four times more affected than women due to the changes in the behavioral and lifestyle patterns [10]. However, high incidence rates are seen amongst the sub populations of women in southern India because of tobacco chewing [13]. Cancer in the tongue is the most common type of cancer and the common site is buccal mucosa and gingiva. In Uttar Pradesh, buccal mucosa or cheek cancer exceeds all the other types of cancer. The incidence of oral cancer in patients who have smoking and tobacco chewing habit is 8.4 times higher than that of patients who did not have that habit. Oral cancer incidence depends on both qualitative and quantitative points of view. A study states that the use of tobacco in the form is smoking has 5.19 times higher risk or precancerous lesion on palate when compared to that of tobacco chewing. States like Uttar Pradesh, Jharkhand and Bihar in India witness more risk of oral cancer [9].

The burdens imposed by cancer vary greatly between the regions of India. Oral cancer incidence and mortality is generally high in affluent States. Oral cancer mortality relates is high due to the mortality in rural areas where cancer treatment facilities are scarce. Poor individuals are also at a higher age-specific mortality risk than are a affluent people. Indian States like Tamil Nadu and Kerala achieve relatively good health outcomes, future health developments will be integrally linked to the nation's economic fortunes and collective commitment to equity and universal health care provision [20]. The use of smokeless tobacco (pan parag, zarda etc) is on rise in north India and especially in states like Uttar Pradesh. The impact of habit leads to high incidence of oral cancer in this region. India's demographic and epidemiological transitions have been slow compared with the progress achieved in the past half century in many other parts of Asia. The population is still fighting for relatively high rates of parasitic, bacterial and viral diseases, while encountering increasing levels of illness caused by cancer [21].

High incidence of oral cancer in India is attributes to a number of etiological factors. Tobacco consumption habit among the patients either as smokeless tobacco or smoking, alcohol consumption is the common causes for oral cancer [22]. Positive family history of oral cancer, viral infections like HPV, poor oral hygiene is the other causes for oral cancer. Based on the TMN classification, 48% of the oral cancer cases were present in the later stages.

Estimates indicate 57% of men and 11% of women between 15-49 years of age use some form of tobacco [18].More than 90% of oral cancer cases report using tobacco products [10]. The forms of tobacco are use of smokeless tobacco, use of betel liquid, pan (pieces of Areca nut), processed or unprocessed tobacco, aqueous calcium hydroxide (slaked lime) and some pieces of a nut wrapped in the leaf of piper betel vine leaf. Additionally, Gutka, Panparak, Zarda, Mawa, Kharra and Khainni. These are dry mixture of powdered tobacco, lime and Areca nut flakes which are chewed or sucked orally. Women chewing tobacco 10 or more times a day have risk 9.2 times that of non-tobacco chewers irrespective of age of initiation of tobacco chewing [10]. Univariate analysis revealed that, in terms of oral dipping products, the risk was 7.3 for consumption of Gutka, 5.3 for consumption of chewing tobacco and 4 for consumption of supari (pure areca nut). However, the lower risk was also found with Mishiri eating [22]. Smoking includes use of cigarettes, Bidi and hookah. These tobacco products are commercially available in sachets or packets and it is very popular among Young a adult which leads to oral cancer in young age. Bidi smokers are 4 times at risk of developing oral cancer compared to nonsmokers [23]. This could be due to poor combustibility as well as the nicotine and tar content of bidi which exceeds that of cigarette [10, 22]. This could be due to poor combustibility as well as the nicotine and tar content of Bidi which exceeds that of cigarette [24]. The number of Bidis smoked per day, a longer duration of smoking and a younger age at starting to smoke was associated with oral cancer.

Drinking alcohol is an important risk factor for oral cancer. Risk increases with number of drinks consumed in a week. A prospective study in India has found that alcohol consumption increases the incidence by 49% among current users and 90% in past drinkers [25]. This could be due to residual effect of alcohol consumption or them having quit the habit due to serious illness. Consumption of alcoholic beverages was associated with increased risk for Oral cancer in men but it was not observed in women because very few women consumed alcohol [10].

Poor oral hygiene also causes oral cancer. In one study, more than 85% of oral cancer patients had poor oral hygiene [12]. Poor oral hygiene related attributable risk is around 32% for men and 64% for women in India. Patients wearing dentures for more than 15 years and not visiting a dentist regularly were highly associated with Oral cancer [26].

The advent of newer imaging techniques and treatment modalities greatly improve the prognosis of the disease. While surgery remains the mainstay of the treatment, radiotherapy (RT) and chemotherapy (CT) is also used as an adjuvant treatment modality. Radiotherapy is used as a primary mode of treatment mainly in carcinomas of base of tongue because tumours are radio responsive and surgery is not advisable due to anatomical barriers. Radiotherapy. Causes oral mucositis, ulceration, dysgeusia and dysphagia. If salivary glands are involved during RT, both structural and functional changes occur in salivary glands. It affects saliva, both qualitatively and quantitatively, making it thick and ropy with decrease in quantity that leads to xerostomia. Irradiation induced oral ulcerations and xerostomia have been reported to facilitate *Candida* growth [27].

MATERIALS AND METHODS

A total of 60 patients have been selected for this study out of these 30 patients samples were taken before treatment and 30 patients samples were taken after treatment. The patient admitted for the diagnosis of oral cancer from January to June 2016 at Rama Medical College Hospital and Research Centre, Kanpur which is a tertiary care centre for health and diagnosis. Oral swabs were collected from all the patients and processed by standard protocol. All isolates were further speculated and antifungal susceptibility was performed according to CLSI guidelines 2016 [28].

A swab of a lesion site is a relatively simple method of detecting growth and semiquantitative estimation of *Candida* was obtained. The sampling approach involves gently rubbing a sterile cotton swab over the lesional tissue and then subsequently inoculating a primary isolation medium such as Sabouraud's dextrose agar (SDA) [29].

The organism was identified using conventional methods that included Gram stain, colony morphology in Sabouraud dextrose agar (SDA) with and without chloramphenicol, Germ tube test, CHROM agar inoculation (HI media), Cornmeal agar with Tween-80 inoculation (HI media). Antifungal susceptibility testing (AFST) was carried out by using disk-diffusion method.

Each isolate was cultured on SDA at 30°C for 48 h. After this, they were seeded on CHROM Agar™ Candida (Chromagar Microbiology) and incubated at 30°C for 48 h. The CHROM Agar™ allows selective yeast isolation, identifying colonies of *C. albicans*, *C. dubliniensis*, *C. tropicalis* and *C. krusei* by morphology and color reaction (30). The strains were identified according to the manufacturer's instructions, which define *C. albicans* or *C. dubliniensis* as green colonies, *C. tropicalis* as steel blue colonies, *C. krusei* colonies as showing rose color and rough aspect, and the other species as developing colonies from white to rose.

Growth at 45°C has been considered a useful test for the differentiation of *C. dubliniensis* (no growth) from *C. albicans* (growth) [31]. This test was used in the 24 positive samples for the germ-tube test, for chlamydo conidia production and that developed green colonies in CHROM Agar™.

Germ Tube Test-0.5 ml of human serum was inoculated with isolated yeast colony. It was incubated for 2 hours at 35°C. After incubation one loopful of yeast inoculated serum was taken on dry, clean slide & covered by cover-slip and observed under low and high magnification for germ tubes. If the test was positive, presumptive identification of *C. albicans* was made [32-36].

Chlamydospore Formation- An isolated colony from the primary culture medium was obtained. The plate of cornmeal agar was inoculated by making three parallel streaks about ½-inch apart at a 45° angle to the culture medium. Formation of large, highly retractile, thick walled, terminal spore was called chlamydospore. The test was used for the identification of *C. albicans* [33-36].

Disk diffusion testing of amphotericin B (20µg), fluconazole (10 µg), Clotrimazole (10 µg) Itraconazole (10 µg) Ketoconazole (10 µg) and Nystin (100 Units) were performed in accordance with CLSI document M44-A3. Agar plates (90 mm in diameter) containing Mueller-Hinton agar supplemented with 2% glucose and 0.5 µg of Methylene blue per ml (GMB) at a depth of 4.0 mm were used. Inoculum was prepared by picking up five distinct colonies of approximately 1mm from 24 hr old growth on Sabouraud's dextrose agar. Colonies were suspended in 5ml of sterile 0.85% saline. The resulting suspension was vortexed and turbidity adjusted to yield 1x 106x 106 cells/ml (0.5 McFarland standard).

The agar surface was inoculated by using a swab dipped in a cell suspension adjusted to the turbidity of a 0.5 McFarland standard. Amphotericin B (10µg), Fluconazole (10µg), Clotrimazole (10 µg) Itraconazole (10 µg) Ketoconazole (10 µg) and Nystin (100 Units), disks (prepared in-house) were placed onto the surfaces of the inoculated plates, and the plates were incubated in air at 35°C to 37°C and read at 18 to 24 hour. Zone diameter is measured to the nearest whole millimeter at the point at which there is prominent reduction of the growth and pinpoint micro colonies at the edge or large colonies within the zone if encountered were ignored.

In vitro antifungal susceptibility testing: The *in vitro* activities of Amphotericin B (10µg), Fluconazole (10µg), Clotrimazole (10 µg) Itraconazole (10 µg) Ketoconazole (10 µg) and Nystin (100 Units) (each procured from Hi-media, Mumbai) was determined by using disk-diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines for antifungal disk diffusion susceptibility testing of yeasts (M44-A2) and results were expressed as susceptible, susceptible-dose dependent and resistant as per ne ameter Interpretive Standards-LSI [37].

Result

Out of total 60 oral cancer patients, 30 patients samples were collected before to radio therapy and 30 patients samples were collected after to radio therapy. Oral swabs were collected from all the patients and processed by standard protocol. Out of 30 samples from pre-treatment cases, 14 were found positive for Candida species and from post treatment cases, 19 were noticed positive. Antifungal susceptibility test result has showed Amphoterocin B was most sensitive drug for all Candida species except *C. parapsilosis* and Fluconazole was found most resistant for all Candida species.

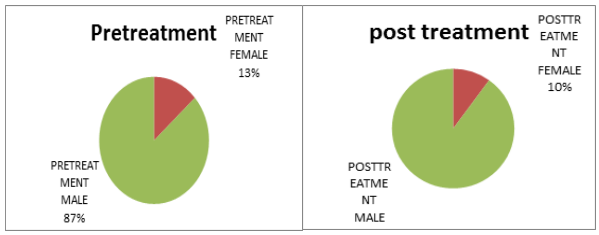
This study was carried out in 30 patients with infections aged of 21 to 90 years. All of the yeast isolates tested grew on CHROMagar Candida medium. After 24 hours of incubation at 37°C, the majority of yeasts had grown well, forming colonies of 1 to 5 mm in diameter; however, growth and colony color development were inconsistent after 24 hours of incubation, and color readings were therefore made only after 48 hours of incubation, as specified in the manufacturer's instructions.

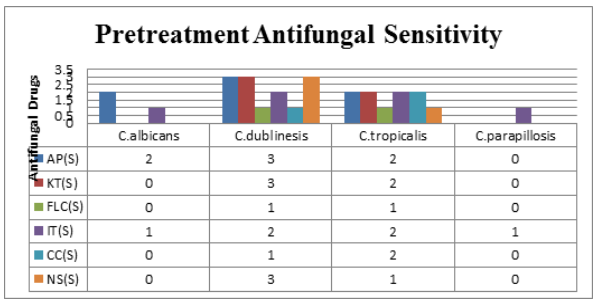
The result of this study shows that CHROMagar Candida was easily identified four important species of Candida on the basis of colonial color and morphology, and was able to accurately differentiate between them i.e. *Candida albicans, Candida glabrata Candida tropicalis* and *Candida krusei.* The majority of *Candida* species amongst the Candida isolates were *Candida albicans* (70%), followed by *C. glabrata* (16.6%), *C. krusei* (6.7%) and *C. tropicalis* (6.7%).

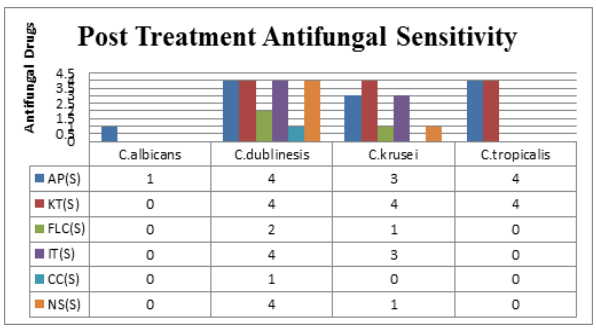
In this investigation, all the isolates of *Candida albicans* were formed light green to green colonies on CHROMagar while *Candida glabrata* isolates were formed pink with a darker mauve center coloured colonies on CHROMagar Candida (Fig. 4). After 48 hours, *Candida krusei* colonies were noted easily distinguishable from those of other yeasts that formed smooth, brownish pink to brownish purple colonies on CHROMagar Candida (Fig. 2 and 3). *Candida tropicalis* isolates all developed a distinctive dark blue gray central color after 48 hours of incubation (Fig. 4). *Candida* species were isolated from 21 male patients and only from 9 female patients. The highest rate of isolation of Candida was found between the age of 50 and 90.

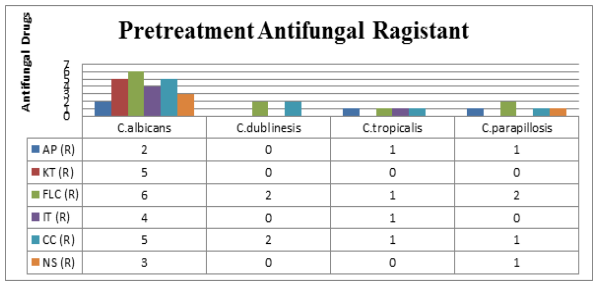
Pre-Treatment patients Sex	No. of patient recorded
Female	4
Male	26
Total	30

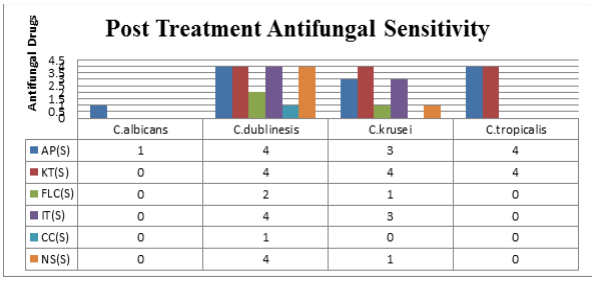
Post-treatment patients sex	No. of patient recorded
Female	3
Male	27
Total	30



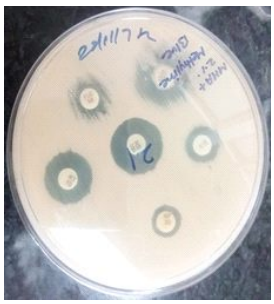








Antifungal sensitivity



Candida spp. grown on Hichrome

DISCUSSION

Oropharyngeal candidiasis is a common fungal infection in cancer patient and currently ranks as the most common human fungal disease. Cytotoxic chemotherapy, radiation or malignancy per se in these patients can lead to compromised cell mediated immunity; something that normally keeps fungal infections in check. Little is known about the epidemiology of oral Candida colonization and infection in developing countries. The present study was carried out to compare the epidemiology of oral Candida colonization and candidiasis in different cancer groups in our state and perform antifungal susceptibility of the isolates recovered. The study compares yeast colonization and infection in three major cancer groups' namely solid tumors, hematological and head and neck malignancies.

A significantly higher rate of total colonization and oral candidiasis was seen in patients receiving chemotherapy and radiotherapy together as compared to patients receiving chemotherapy alone in this study. It is well known that radiotherapy leads to mucositis, xerostomia and mucosal damage, which promote yeast infection. Also, neutropenia due to prolonged chemotherapy, disruption of mucosal barrier and overall damage to cell mediated immunity increases the risk of infection. Similar trends have been seen in various studies conducted across the world. Amador et al. found that radiotherapy induced hypo salivation encourages oral candida colonization that often leads to oral/pharyngeal candidiasis similarly found an increased incidence of oropharyngeal candidiasis in patients receiving concomitant radiotherapy and chemotherapy.

The total numbers of 30 Candida species were isolated in the oral cavity. *Candida albicans* was found to be the predominant with 70% (21/30). The majority of yeast isolates from oral cavity swabs were *C. albicans* (70%), but it was often recovered in association with other yeasts. This was followed by *C. glabrata* 16.6% (5/30), *C. krusei* 6.7% (2/30) and *C. tropicalis* 6.7% (2/30) (Table 1). *C. albicans* 54%, *C.glabrata* 15%, *C.parapsilosis* 12%, *C. tropicalis* 9% and *C. krusei* 3%. of distribution of species. CHROMagar candida is one of the most widely used media in the mycology laboratory. Colony characteristics presented in **Table 1** for identification of *C. albicans*, *C. glabrata, C. krusei* and *C. tropicalis* using CHROMagar. The sensitivity and specificity of CHROMagar andida media for identifying *C. krusei* and *C. tropicalis* were over 99 %. However, other Candida species C. *glabrata* also produce Pink with a darker mauve center coloured colonies on CHROMagar. In the present study, the isolation rates of Candida species is high in ages ranging from 41-80 years old found the isolation rates of Candida species to be high in ages ranging from 60-80 years.

Conclusion

In conclusion oral colonization and infection by Candida spp. is a matter of concern in patients with various malignancies in our hospital. Multiple risk factors contribute to such a scenario in this vulnerable group. *C. albicans* continues to be the number one cause of oral candidiasis in cancer patients in our hospital. All the *C. albicans* recovered were found to be sensitive to the first line azoles; fluconazole and voriconazole. A high resistance pattern in non-albicans spp. to fluconazole seen in this study highlights the need for prompt identification and drug susceptibility testing of the infecting Candida spp. in cancer patients before starting empirical therapy. Also, in view of the increasing reports of resistance to first line azole antifungals, from many parts of the world, even in *C. albicans*, the need for closely monitoring the trends in the epidemiology and drug susceptibility of Candida spp. cannot be overemphasized.

Candida albicans was predominant isolates in oral cancer patients in pre-radiotherapy pateints. The most effective antifungal drug was observed amphoterocin B and least effective antifungal drug was found fluconazole. This study showed a lot of variation in drug sensitivity, this shows irrational use of antifungal drugs. Thus, fungal culture and anti fungal susceptibility tests are necessary for effective treatment. After radiotherapy Candida infection was recorded more than that of pre radio therapy, it shows radio therapy increases the risk of local Candida infection due to immune-suppression. *C. albicans* is the most frequently isolated yeast from the oral cavity infection patients. CHROMagar Candida is a useful culture medium for the isolation and direct identification of *Candida* species, especially *Candida albicans, Candida glabrata, Candida krusei* and *Candida tropicalis*. Easy to prepare, with low cost, CHROMagar Candida proves to be a useful medium for the identification of species of yeast that are isolated with greater frequency in clinical material and for the identification of mixed cultures.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this study.

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