Original Article

DOI: 10.7860/JCDR/2022/55185.16584

Dentistry Section

Rose Bengal Staining, Microbiopsy and Scalpel Biopsy as a Cytology-cum-Histopathology based Diagnostic Scheme for Oral Dysplasias: A Pilot Study

SHILPA DUTTA MALIK[1], UPENDER MALIK[2], MK SUNIL[3]

ABSTRACT

Introduction: Most Oral Squamous Cell Carcinoma (OSCC) is preceded by Oral Potentially Malignant Disorders (OPMDS). These disorders if diagnosed early can be prevented from converting into full blown malignancies. This points towards an ever increasing need for a more accurate, less invasive diagnostic tool to detect these lesions early.

Aim: To analyse and compare the accuracy of 1% Rose Bengal (RB) dye and oral microbiopsies (using dermatologic ring curettes) with conventional scalpel biopsy in diagnosing oral epithelial dysplasias.

Materials and Methods: This cross-sectional pilot study, included a total number of 26 male patients, age range between 40-60 years old, with oral white and red mucosal lesions attending the Outpatient Department (OPD) of Seema Dental College and Hospital, Rishikesh, Uttrakhand, India. After thorough clinical examination, 1% RB staining, microbiopsy, and scalpel biopsy was performed on all included participants. Parameters assessed included clinical signs indicative of dysplasia in red and white lesions such as increase thickness, nodularity, atrophic mucosa, erosion, ulcers and change in colour of mucosa with positive history of tobacco smoking. Hyperchromatic areas owing to increase stain intake (due to increased nuclear cytoplasmic ratio) was obtained after staning with RB dye and histopathological indicators of epithelial dysplasia (cellular and architectural changes) were observed after both microbiopsy and scalpel biopsy. Chi-square test was done to compare results of 1% RB staining and microbiopsies with scalpel biopsies.

Results: Total 26 male patients were included with a mean age of 50 years. There was no statistically significant difference in the accuracy of microbiopsies (p-value=0.913) and 1% RB dye (p-value=0.393) as compared to conventional scalpel biopsy in delineating the epithelial dysplastic changes associated with OPMDS.

Conclusion: Oral microbiopsy, is a relatively novel, accurate, and less invasive diagnostic tool. It has proved to be as effective as scalpel biopsy in diagnosing and grading epithelial dysplasia. Through this article, it was proposed that 1% RB staining, microbiopsy, and scalpel biopsy can be used in conjugation as a part of cytology-cum-histopathology based diagnostic scheme for oral clinically suspicious lesions.

Keywords: First line diagnosis, Live staining, Oral cancer, Potentially malignant disorders

INTRODUCTION

The Oral Squamous Cell Carcinoma (OSCC) accounts for 94% of all oral malignancies and is amongst the ten most frequent malignant neoplasms affecting mankind [1]. In India, OSCC is amongst the top three cancer types [2] and India has been regarded as a global epicenter killing about million patients per year. OSCC has a considerably high incidence, and its associated morbidity and mortality has driven scientists globally to devise methods for its early diagnosis [3].

About 80% of OSCC patients are reported to be preceded by OPMDS [4]. Nevertheless, it has been documented that the malignant transformation for OPMDS is below 18% [5]. Contrastingly, most of OSCCs which are preceded with OPMDS may not appear clinically suspicious on routine oral examination. Diagnostic delay in these lesions can be countered if a robust and effective diagnostic test to rule out if epithelial dysplasia or carcinoma is present [6].

The distinguishing feature between cancer cells and somatic cells is their capacity to multiply [7]. However, the quantum of cellular alterations in OPMDS and their sequel varies. These cellular level alterations may not match with the clinical oral presentation of the lesion. A lot of diagnostic dilemmas in the context of oral epithelial dysplasia associated with OPMDS and OSCCs exists as OPMDS are highly heterogeneous in appearance and may resemble a number of benign or reactive conditions. Hence, many sensitive and predictive techniques along with incisional biopsy have been introduced for the detection of intercellular and intracellular alterations present in oral epithelial dysplasia. Incisional biopsy has been considered as the golden standard diagnostic technique for oral mucosal lesions [8].

However scientific world in the interest of public oral health is always in search of accurate, less invasive diagnostic tools for early detection of changes occurring at both microscopic and macroscopic levels. Many diagnostic techniques can be used in conjugation or as an adjunct to incisional biopsy for screening or improving the efficacy and accuracy of diagnosis. These diagnostic techniques include live staining methods like 1% RB staining. and novel innovative cytological-cum-histological technique like microbiopsies, in the diagnosis of OPMD. These techniques are minimally invasive and can be performed by general practitioner without much of expertise having comparable accuracy and can also be performed in patients where an incisional biopsy is contraindicated i.e., in the case of medically compromised patients, including those with severe or poorly controlled systemic diseases such as coronary artery disease, renal or hepatic impairment, and various endocrinopathies and immune-compromised states, other vascular abnormaliites, or apprehensive patients which are hesitant to undergo incisional biopsy [8].

Thus, the present study was designed to analyse and compare the accuracy of vital staining (using 1% RB dye) and oral microbiopsy (using dermatologic ring curettes) with conventional scalpel biopsy diagnosing oral epithelial dysplasias. Null hypothesis was that

there is no difference between the efficacy of the vital staining and microbiopsy in early detection of oral cancer.

MATERIALS AND METHODS

The present cross-sectional pilot study was performed on a small population, to check the efficacy of microbiopsy and Rose Bengal vital staining, in delineating the epithelial changes. After obtaining ethical approval based on the Helsinki Declaration, written informed consent was taken from all participants and included 26 male patients who attented the OPD of Seema Dental College and Hospital, Rishikesh, Uttrakhand, India. Duration of the study was from December 2014-June 2015.

Inclusion criteria: All the participants who had a provisional clinical diagnosis of oral white and red mucosal lesions with a positive history of tobacco chewing and/or smoking were included in the study.

Exclusion criteria: Subjects with any known allergy to RB dye or a history of systemic conditions like bleeding disorders were excluded from the study.

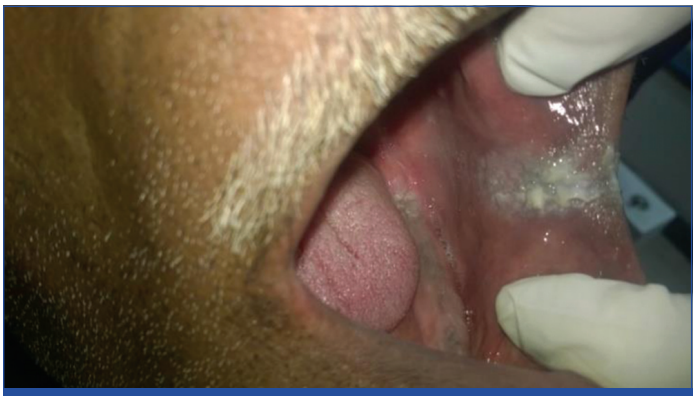
Study Procedure

Parameters assessed were the clinical indicators of dysplasia (ulcers, nodularity, erosion, increase thickness and change in color of mucosa with positive history of tobacco smoking) hyperchromatic areas (which indicates increased nuclear cytoplasmic ratio) obtained after staining with RB dye and histopathological indicators of epithelial dysplasia (cellular and architectural changes) [12]. Observed after both microbiopsy and scalpel biopsy.

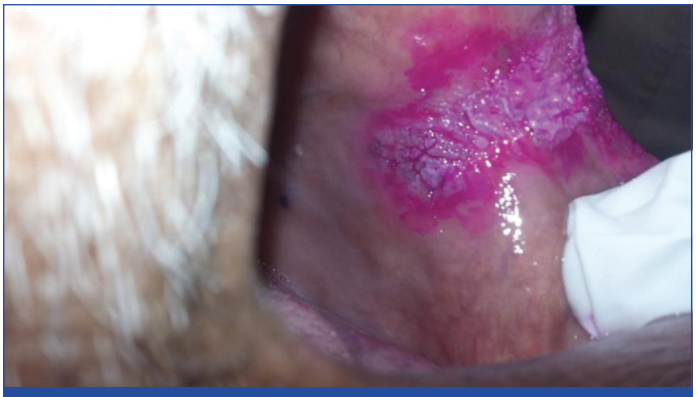
Each patient enrolled in the study was subjected to the following Clinical Procedures (CP):

CP 1: Thorough intraoral clinical examination for the location of the most representative mucosal site for the white and red lesions [Table/Fig-1].

CP 2: Vital staining using 1% RB dye [Table/Fig-2]: Patients were made to rinse their mouth using distilled water for one minute, followed by application of RB solution (using 1% RB dye which was prepared using 1 gm of RB powder in 100 mL of distilled water) for two minutes over the site assessed clinically. Patients were asked to rinse their mouth for one minute with distilled water to remove excess RB dye solution. Intraoral examination of the lesional area stained was carried out, and the intensity of the staining was evaluated visually as mild, moderate, intense, and negative [9].



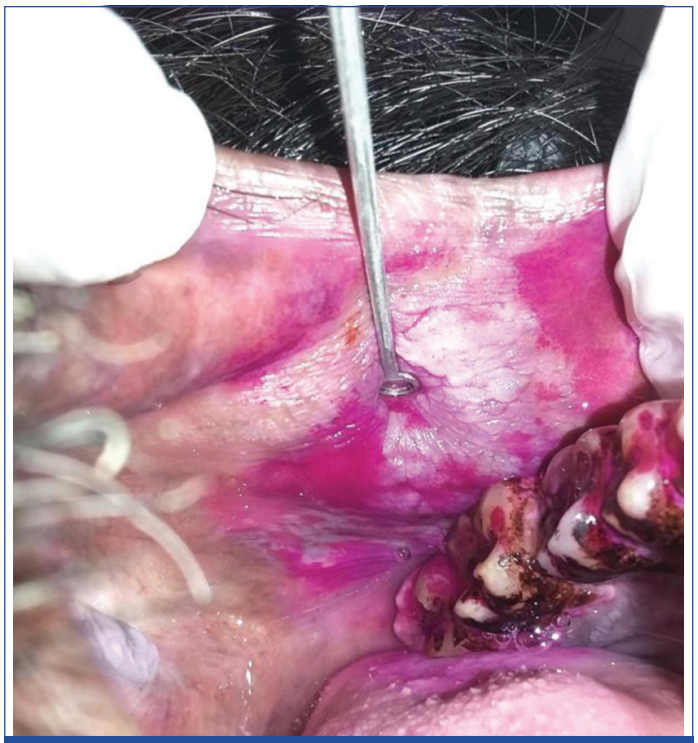
[Table/Fig-1]: Site of the mucosal lesion.



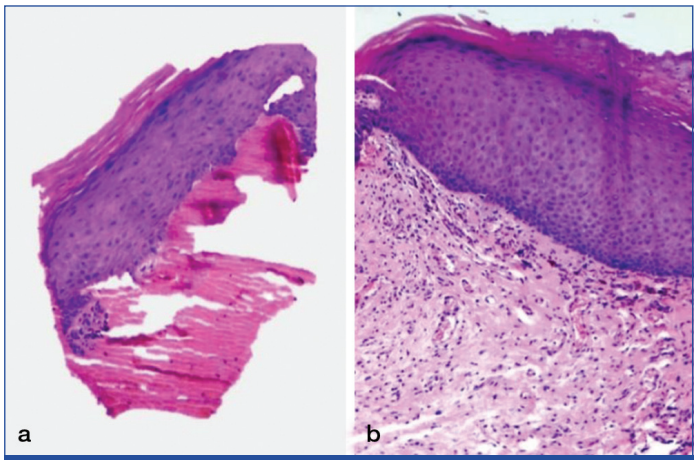
[Table/Fig-2]: The lesional area with 1% RB vital staining.

CP 3: Oral microbiopsy using dermatological ring curettes at the same site [Table/Fig-3]: Microbiopsy using dermatological ring curette with a 3 mm diameter of the intensely stained region by scrapping the lesional area gently by the sharp edge of the curette to obtain pinpoint mucosal bleeding to ensure that basal layer had been sampled. Multiple fragments of tissues thus available were routinely processed, embedded, and sectioned for routine Haematoxylin and Eosin (H&E) staining [Table/Fig-4a]. The sections were coded randomly and observed under a light microscope. The epithelial dysplasia was graded using criteria given by Banoczy J and Csiba A [12].

CP 4: Conventional scalpel biopsy at the same site: Scalpel biopsies were then performed from the stained area of RB dye under local anesthesia infiltration with adrenaline and were processed for regular H&E staining [Table/Fig-4b]. The observer was blinded to the result of vital staining and scalpel biopsy.



[Table/Fig-3]: Microbiopsy using dermatological ring curettes.



[Table/Fig-4]: a) Haematoxylin and Eosin stained picture of microbiopsy (4x); b) Haematoxylin and Eosin stained picture of scalpel biopsy (10x).

STATISTICAL ANALYSIS

Chi-square test was done to compare results of 1% RB staining and microbiopsies with scalpel biopsies. The frequency and percentage

was calculated for the descriptive statistics and the chi-square test with Yates correction was used for the inferential statistics using Statistical Package of the Social Sciences (SPSS) version 21.0. Level of significance for the present study is 5%.

RESULTS

Total 26 cases were included in the present study, age ranging between 40 to 60 years male patients, with mean age of 50 years. Out of 26 cases, 24 cases were diagnosed with hyperkeratosis and epithelial dysplasia (mild, moderate and severe) on scalpel biopsy, 22 cases and 20 cases showed similar results on microbiopsy and vital staining, respectively. The specificity and sensitivity of the RB stain and microbiopsy using dermatological ring curette was calculated as compared to scalpel biopsy and it was observed that microbiopsy was 90.91% sensitive and RB stain was 83.33% sensitive while RB stain showed more specificity of 100% than for microscopy i.e., 50% [Table/Fig-5].

Parameters	RB Staining	Microbiopsy
Sensitivity	83.33%	90.91%
Specificity	100%	50%
Poistive Predictive value	100%	90.91%
Negative Predictive value	33.33%	50%
Diagnostic Accuracy	84.62%	84.62%

[Table/Fig-5]: The specificity and sensitivity of the RB stain and microbiopsy using dermatological ring curette can be calculated as compared to scalpel biopsy.

The diagnostic accuracy was also compared and it was observed that both the techniques (RB stain and microbiopsy) were equally efficient in diagnosing OPMDS. On comparing vital staining with scalpel biopsy using the chi-square test, the p-value was non significant (p-value=0.393) as shown in [Table/Fig-6]. While comparing microbiopsy with scalpel biopsy using the chi-square test, the p-value was non significant (p-value=0.913) as shown in [Table/Fig-7]. Both the procedures i.e., vital staining using 1% RB dye and scalpel biopsy were equally efficient in diagnosing dysplasia.

Incisonal biopsy (26 cases)	Vital staining (26 cases)					
	Mild positive N=8	Moderate positive N=10	Intense positive N=2	Negative staining N=6	Chi-square value	p-value
Hyperkeratosis (10)	4 (40%)	0	2 (20%)	4 (40%)	12.700	0.393
Mild dysplasia (4)	2 (50%)	2 (50%)	0	0		
Moderate dysplasia (6)	2 (33.3%)	4 (66.6%)	0	0		
Severe dysplasia (4)	0	4 (100%)	0	0		
Tissue insufficient (2)	0	0	0	2 (100%)		

[Table/Fig-6]: The comparison between vital staining and scalpel biopsy with the statistical analysis.

Histopathological diagnosis	Scalpel biopsy (26 cases)	Microbiopsy (26 cases)	Chi-square value	p-value
Hyperkeratosis	10 (38.46%)	08 (30.77%)	0.9778	0.913
Mild dysplasia	04 (15.39%)	06 (23.08%)		
Moderate dysplasia	06 (23.07%)	06 (23.08%)		
Severe dysplasia	04 (15.39%)	02 (7.69%)		
Tissue insufficient	02 (7.69%)	04 (15.38%)		

[Table/Fig-7]: The comparison between microbiopsy and scalpel biopsy with the statistical analysis.

DISCUSSION

The outcomes of the present study has proved that both the techniques i.e. vital staining and microbiopsy using dermatological ring curette were equally effective in diagnosing oral epithelial dysplasias, hence, the null hypothesis was accepted. The results of this study were in accordance with Mittal N et al., and Du G-F et al., who proved the efficacy of rose bangal dye in early detection of OPMDS [9,10]. In present study, 1% RB staining, microbiopsies, and scalpal biopsies were performed concomitantly on the same lesion, so that there was no time lapse or positional alterations.

In the Indian scenario, it has been reported in the oral cancer context that the male to female ratio in the urban population is 2:1 and the rural population 5:1 [13]. More incidence of OSCCs can be attributed to the fact that higher rates of smokers are found amongst the Indian male population (30%) as compared to the female population (3%) [14]. The current study was able to enroll only male patients. This biased sample enrolled can be due to two reasons. Firstly, higher rates of smokers are found among the male population in India. and secondly because only male patients with white and red mucosal lesions gave a history of smoking as females must have been hesitant to give the same history dues to social reasons [14].

Though scalpel biopsy is considered as the golden standard diagnostic technique for definitive diagnosis of OMPDS and OSCCs [8]. However, biopsy itself carries many technical problems. Pentenero M have compiled the following pitfalls: [8]. Surgical skills and training required by dentist to perform a biopsy; to choose between incision or excisional type of biopsy; site selection of biopsy; and problems in histopathological interpretation. To overcome these problems, non technique sensitive, non invasive, robust, reliable, painless, budget-friendly, and repeatable techniques are required for screening and as an adjunct to scalpal biopsy [8].

To counter these problems, vital staining, cytological staining, RB (i.e. 4,5,6,7-tetrachloro-2,4,5,7-tetraiododerivative of fluorescein) has been suggested as a marker for alterations in ocular surface physiology [15,16]. RB staining has shown better results than toluidine blue in diagnosing OPMDS [10]. hence it was the choice of vital stain to be tested in present study. Moreover, in the detection of OPMDS, various studies on RB stainings have found sensitivity and diagnostic accuracy to be 90% and 93.9% [9,10]. Present study also showed similar results as the specificity and sensitivity was found to be 83.33% and 100% respectively.

Conventional oral diagnostic cytology has flaws of giving false negative results and inadequate sample cells/depth. Microbiopsy is comparatively advantageous since it is a cytology-cum-histopathology based diagnostic method [8]. Most of the microbiopsies taken in the present study were under infiltrative local anesthesia since it was a relatively less painless procedure as compared to scalpel biopsy. Moreover, the lesion did not bleed much so was easily manageable and did not require suturing unlike the case of scalpal biopsy. Microbiopsies have been positively tested for deriving the first-level diagnosis of oral lesions [8,11]. A previous study comparing microbiopsy with incisional biopsy has found high sensitivity (97.65%) of microbiopsy [17]. These results are in line with our results which demonstrated that sensitivity of microbiopsy was 90.91%. Deliberately results of vital staining with microbiopsy were not compared, since both are unrelated techniques. 1% RB staining is more of a chair side procedure, whereas microbiopsy is a cytology-cum-histopathology based diagnostic method.

Limitation(s)

The limitations of the present study were obvious, i.e. small sample size and male biased sample. The current study is a pilot sample based study so, should be attempted with a larger sample. Evenly distributed gender based samples should be attempted for knowing sex based comparisons of diagnostic challenges in dysplasia associated with OPMDS and OSCCs.

CONCLUSION(S)

The results of the present study showed that both the procedures, i.e., vital staining using 1% RB dye and microbiopsies are very

effective in delineating the dysplastic changes associated with OPMDS and OSCCs. The diagnostic accuracy of rose Bengal dye and microbiopsy obtained to be 84.62% in the present study. The present study points that oral microbiopsy is as effective in diagnosing dysplasia as scalpel biopsy. However, studies with a larger sample size are required to further substantiate the results and findings of the pilot study. The present study results point that all three (i.e. 1% RB staining, microbiopsy, and scalpel biopsy) can be used in conjugation, as a part of cytology-cum-histopathology based diagnostic scheme for oral clinically suspicious lesions.

REFERENCES

[1] Mostafazadeh S, Emamverdizadeh P, Abdal K, Forghani SS. A comparative study of the frequency of myofibroblasts and macrophages between the oral and cutaneous squamous cell carcinoma. J Dent Res Dent Clin Dent Prospects. 2019;13:253-57.
[2] Sankaranarayanan R, Ramadas K, Thomas G, Muwonge R, Thara S, Mathew B, et al. Effect of screening on oral cancer mortality in Kerala, India: A cluster-randomised controlled trial. Lancet. 2005;365:1927-33.
[3] Jha P, Jacob B, Gajalakshmi V, Gupta PC, Dhingra N, Kumar R, et al. A nationally representative case-control study of smoking and death in India. N Engl J Med. 2008;358:1137-47.
[4] Gupta PC, Bhonsle RB, Murti PR, Daftary DK, Mehta FS, Pindborg JJ. An epidemiologic assessment of cancer risk in oral precancerous lesions in India with special reference to nodular leukoplakia. Cancer. 1989;63:2247-52.
[5] Reibel J. Prognosis of oral pre-malignant lesions: Significance of clinical, histopathological, and molecular biological characteristics. Crit Rev Oral Biol Med. 2003;14:47-62.
[6] Warnakulasuriya S. Clinical features and presentation of oral potentially malignant disorders. Oral Surg Oral Med Oral Pathol Oral Radiol. 2018;125:582-90.
[7] Popli DB, Sircar K, Chowdhry A. Telomerase: An exploration toward the end of cancer. Indian J Dent Res. 2017;28:574-84.
[8] Pentenero M, Marino R, Tempia Valenta G, Navone R, Gandolfo S. Microbiopsy a novel sampling technique to early detect dysplastic/malignant alterations in oral mucosal lesions: Practicability by general dentists. J Oral Pathol Med. 2014;43:435-40.
[9] Mittal N, Palaskar S, Shankari M. Rose Bengal staining- diagnostic aid for potentially malignant and malignant disorders: A pilot study. Indian J Dent Res. 2012;23:561-64.
[10] Du GF, Li CZ, Chen HZ, Chen XM, Xiao Q, Cao ZG, et al. Rose bengal staining in detection of oral precancerous and malignant lesions with colorimetric evaluation: A pilot study. Int J Cancer. 2007;120:1958-63.
[11] Pentenero M, Val M, Rosso S, Gandolfo S. Microbiopsy a first-level diagnostic test to rule out oral dysplasia or carcinoma in general dental practice. Oral Dis. 2018;24:109-11.
[12] Bánóczy J, Csiba A. Occurrence of epithelial dysplasia in oral leukoplakia. Analysisand follow-up study of 12 cases. Oral Surg Oral Med Oral Pathol. 1976;42:766-74.
[13] Borse V, Konwar AN, Buragohain P. Oral cancer diagnosis and perspectives in India. Sensors International. 2020;1:100046.
[14] Adult Smokers [Internet]. [cited 2021 Jul 14]. Available from: http://www.china-europe-usa.com/level_4_data/hum/013_4.htm.
[15] Kim J. The use of vital dyes in corneal disease. Curr Opin Ophthalmol. 2000;11:241-47.
[16] Khan-Lim D, Berry M. Still confused about rose bengal? Curr Eye Res. 2004;29:311-17.
[17] Navone R, Pentenero M, Rostan I, Burlo P, Marsico A, Broccoletti R, et al. Oral potentially malignant lesions: First-level micro-histological diagnosis from tissue fragments sampled in liquid-based diagnostic cytology. J Oral Pathol Med. 2008;37:358-63.

PARTICULARS OF CONTRIBUTORS:
1. Associate Professor, Department of Oral Pathology, Teerthanker Mahaveer Dental College, Moradabad, Uttar Pradesh, India; PhD 1st Year Student, Department of Orthodontics and Dentofacial Orthtopedics, Teerthanker Mahaveer Dental College, Moradabad, Uttar Pradesh, India.
2. Professor, Department of Oral Medicine and Radiology, Teerthanker Mahaveer Dental College, Moradabad, Uttar Pradesh, India.
3. Professor and Head, Department of Oral Medicine and Radiology, Teerthanker Mahaveer Dental College, Moradabad, Utter Pradesh, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Shilpa Dutta Malik,
Delhi Road, Moradabad, Uttar Pradesh, India.
E-mail: jbnjmd@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jan 28, 2022
- Manual Googling: Apr 06, 2022
- iThenticate Software: Jun 18, 2022 (5%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Jan 25, 2022**
Date of Peer Review: **Feb 28, 2022**
Date of Acceptance: **Apr 12, 2022**
Date of Publishing: **Jul 01, 2022**