Comparative clinical evaluation of xenograft (Cerabone) versus allograft combined with platelet-rich fibrin for treatment of grade II mandibular furcation defects

Suhina Mitra¹, Deepa G. Kamath¹∗, Nishmitha D. Shetty¹, Srikant Natarajan²

¹. Department of Periodontology, Manipal College of Dental Sciences, Mangalore, Manipal Academy of Higher Education, Manipal, Karnataka, 575001, India
². Department of Oral Pathology, Manipal College of Dental Sciences, Mangalore, Manipal Academy of Higher Education, Manipal, Karnataka, 575001, India

Aim: The present study aims to evaluate the efficacy of naturally-derived bovine hydroxyapatite (Cerabone) versus demineralized freeze-dried bone allograft both combined with platelet-rich fibrin for treatment of grade II mandibular furcation defects. Method: This clinical study included 20 systemically healthy patients, with grade II mandibular furcation defects, performed over 6 months. Control group comprised of open flap debridement + demineralized freeze-dried bone allograft + platelet-rich fibrin and test group comprised of open flap debridement + Cerabone + platelet-rich fibrin. Clinical parameters included: Plaque index, Modified Sulcular bleeding index, Vertical probing pocket depth, Horizontal probing depth, Probing clinical attachment level, Radiographic furcation depth, and radiographic bone fill percentage. Results: Both groups showed satisfactory bone regeneration and improvement in clinical parameters. The test group exhibited greater reduction in vertical probing pocket depth, horizontal probing depth, and higher radiographic bone fill percentage when compared to control group, although these findings were not statistically significant. Conclusion: Both bone grafts were equally effective in treatment of grade II furcation defects. Further long-term studies are required to explore their maximum regenerative potential.

Keywords: periodontal regeneration, demineralized freeze-dried bone allograft, naturally derived bovine hydroxyapatite, furcation defects, platelet rich fibrin

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Introduction

Periodontal regeneration involves the formation of multiple tissues including cementum, periodontal ligament, and bone. Open flap debridement (OFD) alone may not be sufficient for treating periodontal disease. Introduction of bone grafts can enhance clinical results through clinical attachment gain and pocket depth reduction [1], by facilitating the generation of cementum, periodontal ligament, and the alveolar bone through bone-forming cells (osteoneogenesis), laying down scaffold for the formation of bone (osteoaduction), and due to bone inducing properties (osteoinduction) [2].

Cerabone, a xenograftic material, is generated from the ‘mineral phase’ of bovine bone, which displays great similarities to human bone concerning porosity, chemical composition, and surface texture [3]. Due to its unique production method, based on high-temperature heating (>1200°C), the material is completely devoid of organic substances, prions, and possibly antigenic components, thus lowering the risk of immunological responses and disease transmission [4].

Blood and serum proteins are quickly absorbed and stored in the porous three-dimensional network, thus acting as a reservoir for proteins and growth factors. The capillary effect speeds up blood absorption in micro-pores whereas macro-pores promote angiogenesis and rapid ingrowth of osteoblasts [4]. High hydrophilicity, owing to interconnected pores and a rough surface texture, makes it easy to blend with liquids [5].

Combining platelet-rich fibrin (PRF) with bone grafts in a variety of regenerative surgeries is well-documented in the literature [6-9]. Nevertheless, inadequate evidence is available comparing the clinical efficacy of naturally derived bovine hydroxyapatite with the PRF for the treatment of furcation defects.

Therefore, this study aims to compare the treatment outcomes of naturally derived bovine hydroxyapatite, Cerabone (Botiss Biomaterials, Germany), with PRF versus demineralized freeze-dried bone allograft (DFDBA) with PRF, both clinically and radiographically, for treating the grade II furcation defects in mandible.

Material and methods

Study design

This prospective, parallel design study comprised 20 patients with grade II mandibular furcation defects, recruited from the Out-Patient Department of Periodontology, Manipal College of Dental Sciences, Mangalore. This study was performed after obtaining ethical clearance from the Institutional Ethics Committee of our institution (reference number: 19087).

Study population

A total of twenty patients (eleven females, nine males; mean age 41 years) with grade II mandibular furcation defects satisfying the inclusion criteria were chosen for the
study from the out-patient department of the hospital. Selected patients were allocated randomly to either of the two treatment modalities (by a toss of a coin method). The principal investigator performed all study-related procedures and was blinded to the study groups. One investigator (other than the operator) conducted all the clinical and radiographical measurements.

Patient inclusion criteria
1. Patients who signed the informed consent and agreed to participate in the study.
2. Age ≥ 18 years irrespective of gender
3. Mandibular molars with degree II furcation defects [10]; Class II furcation lesion (non-exposed II, and exposed II) [11]
4. Vertical clinical attachment loss of ≥ 4mm after initial therapy
5. Full mouth plaque index and modified sulcular bleeding index scores: <20%.
6. Adequate gingival biotype of ≥ 1mm

Patient exclusion criteria
1. Systemically compromised patients, contraindicated for any periodontal surgeries.
2. Periodontal surgery performed in the last 24 months in the intended site.
3. Pregnant or lactating mothers.
4. Patients with tobacco consumption habits of any type
5. Third molars with furcation involvement
6. Superficial caries or restorations in the area to be treated
7. Immunocompromised individuals
8. Patients undergoing orthodontic involvement

Sample size
Based on the study by Sezgin et al. published in Brazilian oral research in 2017 showed a clinically significant difference of 2 units [12]. A sample size of 10 units was obtained using the formula:

$$n = \left( \frac{Z_{1-\alpha/2} - Z_{1-\beta}}{\sigma} \right)^2 \cdot \frac{d^2}{\sigma^2}$$

where N is the number of samples,
\(d\) = the minimum difference in the values which will make clinically relevant impact
\(\sigma\) = average standard deviation,
\(Z(1-\alpha/2)\): Z score for the alpha error chosen
\(Z(1-\beta)\): Z score for the power chosen [12]
(Alpha error: 3%, Power of the study: 80%, and a clinically significant difference: 2 units).

After obtaining written consent, data regarding the chief complaint, history of present illness, medical, dental, drug, family, personal history, and gingival as well as periodontal status were recorded in the case proforma.

Patients were examined under good illumination using a mouth mirror, Williams Graduated Periodontal Probe, UNC-15 probe, and cotton gauze.

Initial therapy
Every study participant was subjected to Phase-1 therapy, comprising full mouth scaling and root planning using hand and ultrasonic instruments. Detailed oral hygiene instructions were given and re-evaluation was performed once every 2 weeks. Oral hygiene instructions were reinforced on every follow-up appointment until every patient exhibited good oral hygiene having full mouth plaque index (FMPI) (< 20%) and full mouth modified sulcular bleeding index (FMBI) (< 20%)

Before the surgery
The patients were directed to rinse for a minute using 0.2% chlorhexidine gluconate. After induction of local anesthesia, root planing was performed.

Preparation of PRF
PRF was prepared as per the protocol developed by Choukroun et al. [13], without biochemical manipulation of blood. 10ml blood was withdrawn from the antecubital vein of the patient (Figures 1A and 1B), which was then immediately spun at 3000 rpm for 10 minutes using REMI R-8C Laboratory Centrifuge (Figure 1C) without the use of any anticoagulants. Three fundamental layers were separated by centrifugation: the layer of red cells at the bottom, the layer of acellular platelet-poor plasma at the top, and the layer of the PRF clot sandwiched between the two layers (Figure 1D&E). The middle layer PRF clot (Figure 1F) was then extracted and compressed between two pieces of cotton gauze to produce a membrane (Figure 1G). The PRF membrane was positioned as a guided tissue regeneration membrane over the recipient site.

Surgical procedure
In sites assigned to the test group, OFD was performed with Bovine Xenograft (Cerabone) and PRF, while control group sites were treated with OFD, DFDBA, and PRF.

Test group: OFD + Cerabone + PRF
Under local anesthesia, using a number 15 blade, a sulcular incision was given (Figure 2A) following which a full-thickness flap was reflected retaining sufficient tissue to attain primary closure (Figure 2B). After thorough debridement of the exposed furcation defect and the adjacent bone (Figure 2C), Cerabone (Figure 2D) was then packed and condensed in the furcation defect (Figure 2E). Following this, the defect was covered using a contoured platelet-rich fibrin membrane (Figure 2F) and stabilized using a sling suture. The flap was later adapted and sutured in position with a 5-0 non-resorbable silk suture (Figure 2G). The periodontal dressing was given to cover the surgical site (Figure 2H).
A similar surgical procedure was performed in the control group. The defect was filled using DFDBA and condensed in place (Figure 3 A-D). PRF membrane was placed over the defect (Figure 3E) and stabilized with a sling suture. The mucoperiosteal flap was sutured (Figure 3F) and a periodontal dressing was placed (Figure 3G).

**Post-operative care:**
Patients were given post-operative instructions and analgesic was prescribed to minimize discomfort if any. The patients were instructed to maintain plaque control using chlorhexidine gluconate (0.2%) mouthwash. Suture was removed after two weeks following which they were asked to return at 1-, 3- and 6-month intervals, and oral prophylaxis was carried out. Clinical parameters were noted pre-operatively and at 6 months postoperatively (Figures 4 and 5).

**Statistical analysis**
The parameters of plaque index, modified sulcular bleeding index, vertical and horizontal probing depth, radiographic furcation involvement, and clinical attachment loss were compared between the cases and controls using an independent t-test. The same variables were compared between the baseline and the 6-month values using paired t-tests in
cases and controls separately. The analysis was done using IBM SPSS version-20.0 (IBM Chicago, USA) with a level of significance deemed as 0.05.

**Results**

A total of 20 Grade II furcation defects were treated, with 10 defects in the test group (OFD + CERABONE + PRF) and 10 defects in the control group (OFD + DFDBA + PRF).

A statistically significant difference was not observed between the groups at the baseline data. After the surgical procedure was completed, the clinical parameters were measured again for both groups at 6 months. The inter-group comparison did not reveal any statistically significant difference between the two groups (Table I).

The mean plaque index (PI) scores for the test and control groups at baseline were 1.13±0.33 and 1.1±0.32 and at 6 months were 0.57±0.15 and 0.57±0.22, respectively. A statistically significant difference was obtained for the test group (p-value 0.001) and control group (p-value 0.001). The mean modified sulcular bleeding index (M-SBI) scores for the test and control groups at baseline were 0.58±0.15 and 0.53±0.14 and at 6 months were 0.35±0.09 and 0.34±0.1, respectively. This variance was
statistically significant for the test group (p-value 0.001) and the control group (p-value<0.001). However, the intergroup comparisons did not show any statistically significant difference after 6 months of follow-up for PI (P-value 0.85), and M-SBI (P-value 0.85). The mean vertical probing pocket depth (VPPD) for the test and control groups at baseline were 4.1±0.88mm and 3.7±1.16mm and at 6 months were 2.2±0.42mm and 2.2±0.42mm, respectively. A difference that is statistically significant for the test group (p-value<0.001) and control group (p-value 0.001), was obtained. The mean horizontal probing pocket depth (VPPD) for the test and control groups at baseline were 3.6±0.52mm and 3.5±0.53mm, respectively, and at 6 months, it decreased to 0 in both groups. The mean probing clinical attachment level (PCAL) for the test and control groups at baseline were 5.7±0.67mm and 5.6±0.7mm, and at 6 months were 1.6±0.97mm and 1.5±0.97mm, respectively. The mean radiographic furcation depth (RFD) for the test and control group at baseline were 3.5±0.53mm and 3.6±0.52mm, and at 6 months were 0.2±0.42mm and 0.3±0.48mm, respectively. A difference that is statistically significant for the test group (p-value<0.001) and control group (p-value<0.001), was obtained for VPPD, PCAL, and RFD. Comparison of the radiographic bone fill percentage (RBF gain %) between the two groups statistically non-significant result (p-value 0.62) (Table II).

### Table II. Intergroup and Intragroup comparison at baseline and 6 months

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Mean ± Standard deviation (baseline)</th>
<th>P value</th>
<th>Mean ± Standard deviation (6 months)</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>Plaque index</td>
<td>Test</td>
<td>1.13±0.33</td>
<td>0.838</td>
<td>0.57±0.15</td>
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<td></td>
<td>Control</td>
<td>1.1±0.32</td>
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<td>0.57±0.22</td>
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<tr>
<td>Modified Sulcular Bleeding Index</td>
<td>Test</td>
<td>0.58±0.15</td>
<td>0.403</td>
<td>0.35±0.09</td>
<td>0.851</td>
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<tr>
<td></td>
<td>Control</td>
<td>0.53±0.14</td>
<td></td>
<td>0.34±0.1</td>
<td></td>
</tr>
<tr>
<td>Vertical probing pocket depth (in mm)</td>
<td>Test</td>
<td>4±1.15</td>
<td>0.569</td>
<td>2.2±0.42</td>
<td>1</td>
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<tr>
<td></td>
<td>Control</td>
<td>3.7±1.16</td>
<td></td>
<td>2.2±0.42</td>
<td></td>
</tr>
<tr>
<td>Horizontal probing depth (in mm)</td>
<td>Test</td>
<td>3.6±0.52</td>
<td>0.673</td>
<td>0±0</td>
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<tr>
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<td>Control</td>
<td>3.5±0.53</td>
<td></td>
<td>0±0</td>
<td></td>
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<tr>
<td>Probing clinical attachment level (in mm)</td>
<td>Test</td>
<td>5.7±0.67</td>
<td>0.749</td>
<td>1.6±0.97</td>
<td>0.82</td>
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<tr>
<td></td>
<td>Control</td>
<td>5.6±0.7</td>
<td></td>
<td>1.5±0.97</td>
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</tr>
<tr>
<td>Radiographic furcation depth (in mm)</td>
<td>Test</td>
<td>3.5±0.53</td>
<td>0.673</td>
<td>0.2±0.42</td>
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<td>3.6±0.52</td>
<td></td>
<td>0.3±0.48</td>
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</tbody>
</table>

P < 0.05 considered statistically significant; P > 0.05 considered statistically non-significant.

### Discussion

Periodontal disease may involve alterations in the morphology of the bone, which can be treated using four different hard tissue replacement graft materials including autografts, allografts, xenografts as well as alloplasts. Although, autografts are regarded as the “gold standard”, they have a few limitations, including limited availability of bone volume, donor site morbidity, and the unpredictable rate of replacement (14).

DFDBA, due to its osteoconductive and osteoinductive properties, encourages the development of new attachment
systems in infrabony defects. The bone morphogenetic proteins (BMPs) and growth factors that are exposed after the allograft’s acid demineralization process are thought to be responsible for the osteoinductive property, which allows for rapid revascularization and the ingrowth of hard tissue in osseous deformities, consequently boosting periodontal regeneration [7].

Xenograft is currently an additional choice as a bone graft material. Due to its osteoconductive qualities, this substance promotes bone growth and undergoes remodeling throughout a gradual resorption process [15] This led the authors of the present study to assess the relative efficacy of Cerabone when compared to DFDBA.

DFDBA was considered a positive control for this study since it has consistently shown good results [16]. Studies have shown a statistically significant outcome in favor of a bone graft against a non-grafted location. Hence comparison with a non-graft control was not favored [17,18]. The selection of grade II furcation defects was derived from the information provided by the outcomes of controlled clinical research that grade II furcation allows better stability, increased blood supply to the graft, and better containment [19]. Both materials used in the study were tolerated well by the participants, and these results concur with the findings of Hernandez et al. [20]. The choice of bone graft was the Cerabone since it is considered to possess several superior qualities including enhancement of revascularization and stabilization of clot, better-handling properties, scaffold for the synthesis of new bone, improved osseointegration, and delayed resorption rate [3].

To hasten the osteogenic activity, it is encouraged to incorporate a variety of growth factors, such as transforming growth factor- beta, platelet-derived growth factor (PGF), and proteins. The use of PRF in conjunction with bone transplants is intended to improve density as well as the maturation of bone. When compared to grafts without PGFs, the radiographic rate of maturation of bone grafts with PGFs was seen as 1.6–2.16 times faster [21].

A potent bio-scaffold for exposing osteoblast and gingival fibroblast growth and differentiation is PRF, which combines fibrins and cytokines [22]. Clinical investigations have shown that PRF enhances the regeneration of soft tissue and bone [23], as well as the regeneration of periodontal tissues [24]. Combining PRF with autologous bone or bone substitutes like Bio-Oss can improve its capacity to repair and restore damaged tissues [25]. Hence, PRF has established itself as a highly inductive and bio-compatible scaffold. Taking all these into consideration, this present study aimed to assess the use of Cerabone with PRF and allograft with PRF for the treatment of grade II furcation defects in the mandibular region.

This study was performed over 6 months as a prospective, parallel-design clinical study. At baseline, the considered parameters in both groups did not show any statistical difference, such that the same commencing point is ensured for the procedures. All the parameters evaluated after 6 months were similar when comparing both groups (DFDBA vs Cerabone). The intergroup comparison findings did not show statistical significance. Healing in both the groups progressed as normal satisfactory healing and at 6 months of evaluation, there was a decrease in the vertical probing pocket depth, horizontal probing pocket depth, increase in probing clinical attachment level, radiographic furcation depth, as well as sufficient bone fill achieved in both groups.

The decrease in vertical probing depth in the test group in the present study was statistically significant. A 2mm reduction in VPPD values was seen after 9 months in a study conducted by Birkan et al. [26] by combining PRF with bovine-derived xenograft for treating intrabony periodontal defects. The authors stated that xenografts might improve the space that is maintained for tissue regeneration and promote bone-filling cells. Efficient manipulations and delivery to the surgical areas were made possible by PRF. In a study by Agarwal et al. [8] to ascertain the multiple effects of PRF in combination with a DFDBA in the management of intrabony periodontal abnormalities, PD was reduced statistically significantly.

In the present study, the VPPD reduction in the test group was greater than that of the control group, but this finding was statistically insignificant (p-value 0.51). Similar results were seen in a study carried out by Richardson et al. [27] when particulate bovine-derived xenograft (BDX) was compared to DFDBA for treating the intrabony vertical defects in moderate to severe adult periodontitis. The authors stated that there was a substantial difference in the handling properties of the two materials, with the BDX group showing superior handling properties.

Both the control and test groups in the present study showed a statistically significant reduction in horizontal probing depth. Similar findings were seen in a study by Taheri et al. [28] evaluating BDX with a bioabsorbable collagen membrane for the treatment of grade II furcation defects and by Agarwal et al. [29] evaluating the efficacy of PRF along with DFDBA for the treatment of mandibular degree II furcation defects.

In the present study, the test group shows a greater decrease in the mean reduction of horizontal probing depth after 6 months in comparison to the control group, although this finding is statistically insignificant.

The test group in this present study showed an increase in the probing clinical attachment level. Similar improvement in PCAL was seen in a study by Taheri et al. [28] evaluating the effectiveness of BDX with bioabsorbable collagen membrane. Birkan et al. [26] also obtained similar results in an experiment to evaluate the use of PRF combined with BDX and concluded that regenerative treatment of the intrabony defects using this combination provided favorable healing.

Richardson et al. [27] conducted a study to assess the efficiency between BDX and DFDBA. Although, there was a significant increase in the clinical attachment levels at 6
months in comparison to baseline measurements in both groups, the comparison of treatment between the two graft materials revealed no significant difference. On re-entry after six months, it was revealed that neither group experienced any recession as compared to the baseline.

Another study conducted by Blaggana et al. [15] evaluating the relative efficacy of DFDBA versus bovine bone xenograft (ABBX) for the management of infrabony periodontal defects, showed a substantial increase in the attachment level in both the groups 12 and 24 weeks post-operatively, while improved results were obtained with DFDBA as compared to ABBX. The authors stated that the slow rate of resorption of ABBX, caused the scaffold formation to stay for a longer duration of time thus leading to a more favorable outcome in terms of bone fill. This finding is in contrast with the findings of the present study, and this difference can be attributed to the difference in the type of defect, the number of subjects, and the fact that no additional membrane was used.

A statistically significant reduction was observed in the radiographic furcation depth in the test group as well as the control group at 6 months. Similar results were obtained in a study conducted by Nader et al. [5], where Cerabone was compared to an autogenous bone graft for the treatment of 2- and 3-wall intrabony defects. In another experiment by Kothisiude et al. [30], where a significant decrease in grade II furcation depth was seen in sites treated with xenograft (Bio-Oss) with amniotic membrane after 9 months.

In the present study the radiographic bone fill gain percentage was 2.5% higher in the test group. These results are similar to the findings in the study by Richardson et al. [27] comparing DFDBA vs BDX. On re-entering after six months, both the treatment groups showed enhanced bone-fill, percentage bone-fill, and % defect resolution. The BDX group showed a mean 3.0% higher bone fill and a mean defect improvement of 5% higher than that exhibited by the DFDBA group. The authors attributed these findings to the space maintenance property of BDX.

In another study, by Kollati et al. [3], performed to compare the efficacy of Cerabone and PRF matrix with collagen plug versus unassisted natural healing in sites of extraction, the increase in the percentage of bone fill was seen to be statistically significant at the test site when compared to the control site.

From a surgical standpoint, it is important to note the difference in the handling characteristics of the two materials evaluated. Cerabone exhibited the following favorable handling properties when compared to DFDBA,

1. convenient delivery of the graft to the surgical site
2. enhanced packing and adaptation to the defect,
3. the capacity to exhibit adherence in the defect, even in the presence of hemorrhage, to provide a long-lasting and secured graft stability
4. retention of space following soft tissue closure

The limitations observed in the present study were the small sample size which restricted the statistical analysis of the result, and the short-term analysis limited the evaluation of the stability of periodontal treatment outcome. Histological evaluation could have described the bone type that is formed in the defect site. The use of advanced radiographic techniques, instead of the intraoral periapical radiographs evaluate bone fill, is advised for evaluating the efficacy of Cerabone with PRF for periodontal treatment.

The study revealed that both graft materials enhanced clinical outcomes. After six months, no discernible changes were seen between the two intervention groups in terms of clinical or radiographic characteristics. However, the authors favored the use of Cerabone due to the restricted availability of allograft and its ease of handling.

**Conclusion**

The present study aimed to compare the efficacy of Cerabone with PRF versus DFDBA with PRF in the treatment of grade II furcation defects. Numerous bone grafting materials have been evaluated clinically and radiographically [31]. A successful bone graft heals, gets incorporated, revascularizes, and eventually assumes the form desired. Cerabone is derived from the mineral phase of bovine bone, which shows a strong resemblance to the human bone in its chemical composition, porosity, and surface structure [3]. Its three-dimensional porous network enables a fast penetration and adsorption of blood and serum proteins and serves as a reservoir for proteins and growth factors. Adhesion of proteins and signaling molecules from the blood further improves the biological properties of Cerabone.

Thus, it can be concluded that:

- Both the groups showed satisfactory periodontal regeneration over 6 months, supporting their use in the management of grade II furcation defects
- Both groups showed statistically significant improvement in all the parameters (VPPD, HPD, PCAL, RFD) when compared to the baseline values
- The test group showed a greater reduction in VPPD and HPD, and greater radiographic bone fill percentage when compared to the control group, but these findings were not statistically significant.
- Within the limits of the study, it can be concluded that Cerabone along with PRF may be considered as a predictable, effective, and viable alternative to DFDBA with PRF for the treatment of grade II furcation defects.
- Recognizing the potential benefit of Cerabone in the treatment of furcation defects, further long-term studies with larger sample sizes will be required to give a conclusive statement on the treatment outcome.

**Authors’ contribution**

SM (Conceptualization; Data curation; Investigation; Methodology; Project administration; Validation; Visualization)

DK (Conceptualization; Data curation; Investigation;
Methodology; Project administration; Validation; Supervision
NS (Visualization; Writing- Original draft; Writing- Review and editing)
SN (Formal analysis; Software; Supervision)

References