Enchancing Gingival Biotype using the Wonder Liquid iPRF– A Pilot Study

Swetha A[1], Nikhila Chandramohan[2]

ABSTRACT

Introduction: Thickness of gingiva in the facio-palatal dimension, otherwise called Gingival biotype is a predisposing factor for gingival recession. Gingival recession being one of the most common aesthetic concerns encountered in routine dental practice, an attempt should be made to increase the gingival thickness in order to prevent gingival recession. Injectable-platelet-rich fibrin (i-PRF) is a rich source of autologous growth factors has recently shown favourable results in regenerative dentistry. The aim of this present study was to evaluate the effect of i-PRF on enhancing gingival thickness in individuals with thin periodontal phenotypes.

Material and methods: In this study, 10 systemically healthy patients with thin gingival thickness were treated with i-PRF. i-PRF was injected into the attached gingiva in three sessions with 2 week intervals.

Results: On assessment of gingival thickness, 8 weeks after the final injection, a statistically significant improvement was observed [from 0.68 ± 0.120mm to 1.025 ± 0.013mm]. An average of 51% increase in gingival thickness was achieved.

Conclusion: In individuals with thin periodontal phenotypes, standalone i-PRF may have a positive influence in increasing gingival thickness. The results suggest that application of i-PRF can be considered as a promising non-surgical method for increasing gingival thickness.

Keywords: Gingival Biotype, Injectable Platelet Rich Fibrin (i-PRF), Gingival Thickness, Growth Factors.

INTRODUCTION

In this era of aesthetics-driven dentistry, a pleasing smile encompasses the harmonious relationship between teeth, in its appropriate size, position and shape with surrounding soft tissue. This compatibility of the soft tissue over the hard tissue depends upon a myriad of factors. Among the factors that may impede success in dental treatment, the often neglected gingival biotype (GB) is one of the greatest causes of concern. Different tissue biotypes respond differently to inflammation and to surgical and restorative treatment; consequently, it is crucial to identify tissue biotype before any intervention.

Ochsenbein and Ross first indicated that there were two main types of gingival morphology, namely the *'scalloped and thin'* or '*flat and thick'*; gingival contour closely following the contour of the underlying bone.[1] A more comprehensive term "periodontal biotype" was later introduced by Seibert and Lindhe to categorize the gingiva into *"thick & flat*" and *"thin & scalloped*" biotypes.[2] A gingival thickness (GT) of ≥2 mm was considered thick and a gingival thickness of <1.5 mm was referred as thin tissue biotype. Thick gingival tissue is associated with a broad zone of the keratinized tissue and flat gingival contour suggestive of thick bony architecture and is more resistant to inflammation and trauma. Thin gingival tissue is associated with a thin band of the keratinized tissue, scalloped gingival contour suggestive of thin bony architecture and is more sensitive to inflammation and trauma.[3] According to Weisgold, individuals with a thin, scalloped gingiva demonstrated a greater prevalence of recession.[4]

The thick GB is a significant predictor of clinical outcome of periodontal therapy, root coverage procedures, and implant placement. It was also shown that patients with thin GB were more likely to experience gingival recession following nonsurgical periodontal therapy.[5] Better surgical outcome with thick GB can be attributed to the presence of increased vascularity, greater perfusion with enhanced oxygenation, clearance of toxic products, immune response and growth factor migration. In full/partial thickness flaps, postoperative alveolar bone destruction is thought to be associated with GB. The periodontal phenotype also affects the mucogingival surgical techniques to be selected for root coverage.[6]

Various surgical techniques have been put forth to enhance GT and can result in constructive treatment outcomes. They include the use of subepithelial connective tissue grafts, acellular dermal matrix, chorion membrane and guided tissue regeneration (GTR). However, donor site morbidity, limited availability, treatment costs and increased operating time are some of the associated drawbacks.

Platelet concentrates have been utilized in dentistry for over three decades as a regenerative tool which is capable of releasing supraphysiological doses of growth factors responsible for inducing tissue regeneration derived from autologous sources. PRF is a second generation platelet concentrate which has scored over platelet rich plasma by virtue of its properties, ease of preparation, and cost effectiveness. It consists of a three dimensional fibrin matrix which gradually releases platelet cytokines, platelet derived

[1]Post Graduate, Department of Periodontology, [2]Post Graduate, Department of Periodontology, M R Ambedkar Dental College & Hospital , Bangalore, Karnataka, India

Corresponding author: Dr. Swetha A , #44/1 Yamuna Residency, 3rd Cross Vasanth Nagar, Bangalore -560052, Karnataka, India

How to cite this article: Swetha A, Chandramohan N. Enchancing gingival biotype using the wonder liquid iPRF– a pilot study. International Journal of Contemporary Medical Research 2021;8(8):H5-H9.

DOI: http://dx.doi.org/10.21276/ijcmr.2021.8.8.12

growth factors (PDGF)-α and (PDGF)-β, transforming growth factor beta (TGF)-β and insulin-like growth factor-1 (IGF-1) as it gets is resorbed, aiding the process of healing. These growth factors are known to modulate and up regulate one growth factors function in the presence of second or third growth factor. Shetty et al. in their study demonstrated that placement of PRF membrane over denuded root surface in conjunction with coronally advanced flap results in the improvement in the thickness of gingiva.[7] Dayoub et al in 2019 concluded that use of PRF with tunnel flap is a successful treatment option in modifying thin GB and could serve as an alternative to connective tissue grafts.[8]

Recent development of an injectable liquid formulation of PRF (termed i-PRF) based on low-speed concept for blood centrifugation by Ghanaati et al. has been pursued with the aim of delivering clinicians an easy to use platelet concentrate in liquid formulation which can be either utilized alone or in combination with various biomaterials.[9] Taking advantage of slower and shorter centrifugation speeds, a higher presence of regenerative cells with higher concentrations of growth factors can be observed when compared to other formulations of PRF. Miron et al in his in-vitro study has concluded that i-PRF demonstrated higher leuckocyte concentration, enabling slow and sustained release of growth factors, induced higher fibroblast migration and expression of PDGF, TGF-β, and collagen-1 mRNA[10,11].

In light of these factors, due to the biological potential of i-PRF to boost neoangiogenesis, neocollagenesis and wound healing, this study was designed to evaluate the effects of standalone i-PRF on the GB in individuals with thin biotypes.

MATERIAL AND METHODS

Subjects belonging to both genders were selected from the outpatient department of periodontolgy. Approval from the Ethical Committee of M.R. Ambedkar Dental College and Hospital was obtained. The treatment procedure was explained and written informed consent was obtained from all patients. The inclusion criteria was systemically healthy, non smoker subjects, with thin gingival biotype (thickness ≤1.5mm) in maxillary or mandibular anterior region , absence of periapical lesion in the area under study, age range between 25-55 years, with plaque index[12] and gingival index[13] between zero and one, non-contributory medical history, no use of drugs such as antibiotics, corticoids, chemotherapeutics, or immunological modulators in the past 6 months that might alter the expected response of oral tissues, no use of protective or orthodontic apparatus and presence of > 2mm band of keratinized tissue around the test teeth. Patients with presence of periodontal pocket, attachment loss, malocclusion, alcoholics, who had received any chemotherapeutic mouth rinses and oral irrigation during the past one month, pregnant and lactating patients were excluded from the study.

All volunteers were reinforced with oral hygiene instructions and full-mouth initial periodontal therapy was performed which consisted of scaling and root planning, before the clinical examination to check for any potential gingival inflammation. Patients were screened for thin GB using transgingival probing method at baseline. Follow up measurements were taken at 4, 6 and 8 weeks. Measurement was recorded 3 mm below the gingival margin at the attached gingiva or the alveolar mucosa using a #15 endodontic K-file with a silicone disk stop (Fig 4). The file was inserted perpendicularly from the vestibular midpoint at 3 mm apical of the gingival margin, through the soft tissues until a hard surface was felt. The silicon disc was laid in tight contact with the soft tissue surface. The penetration depth between the silicone disc and the file tip was measured using a digital vernier calliper (Fig 5). Clinical measurements were carried out by a single calibrated examiner to ensure an unbiased evaluation and rule out inter-examiner variability. Injection procedure was performed by the second operator to avoid intraoperator variation.

Treatment procedure

Test site was anesthetized using 2% lignocaine with adrenaline (1:200,000). iPRF was prepared as per the protocol put forth by Chokroun et al.[10] For i-PRF preparation, *two plastic tubes* of 5 ml of whole blood each without anticoagulant were centrifuged at 700 rpm for 3 min (60×g) at room temperature in a Centrifuge. The resulting upper liquid layer (Fig 2) was collected using an *insulin syringe* [0.25mm (31G) x 6mm needle, BD GlideTM needle insulin syringe] as *i-PRF* (Fig 3) and immediately injected into the attached gingiva till the blanching and fullness of gingiva was noted (Fig 6), before the liquid turned into a gel consistency. Since it is autologous, the entire obtained concentrate was injected, without any predetermined quantity. The patients were instructed not to brush at the treated site on the day of treatment, resume one day after, using a non-traumatic roll tooth brushing technique with an ultra-soft toothbrush. At the first follow-up (4 weeks later) the patients did not show a very significant improvement, therefore; we decided to perform two more shots of iPRF injections at the end of 4th week and 6th week (2 week intervals). Follow-up at 8th week was done to assess the sustainability of the obtained results. Being autologous in nature, no side effects were anticipated.

STATISTICAL ANALYSIS

Means and standard errors (SE) were calculated, and data were analyzed for statistical significance and Intra group comparison was done by one way ANOVA test using SPSS software. (SPSS Software, Inc., La Jolla, CA, USA).

RESULTS

A total of 10 patients with a mean age of 37.5± 14.4 yrs were evaluated. *Table 1* shows the demographic distribution in the study group. Among the study subjects 8 were of females and 2 were of males, 7 test sites were in the maxilla and remaining 3 were in the mandible. iPRF injections were uneventful. Slight tenderness at the injection site, was reported and typically lasted for the first 1–2 postoperative days.

The results are presented in Table 2 and Fig 1 and representative photographs are shown in Fig 2-7. Differences

Age & Gender distributions among study subjects			
Variables	**Categories**	**n**	**%**
Age Groups	25-35 yrs	4	40%
	36-45 yrs	4	40%
	46-55 yrs	1	10%
	> 55 yrs	1	10%
Sex	Males	2	20%
	Females	8	80%

Table-1: Demographic data

Intervals	Number	Mean & Std Dev (GT)
Baseline	10	0.68 ± 0.120
Four weeks	10	0.79 ± 0.530
Six weeks	10	0.86 ± 0.050
Eight weeks	10	1.025 ± 0.013

Table-2: Results

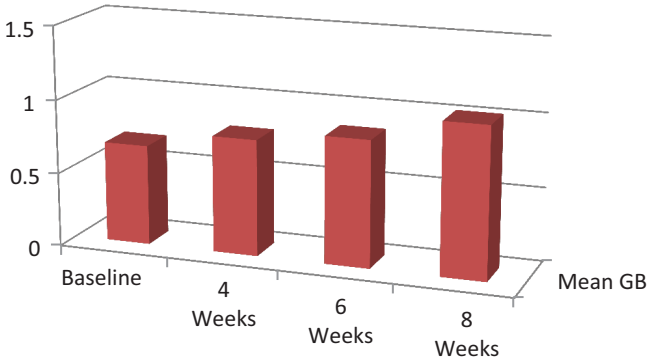

Figure-1: Results

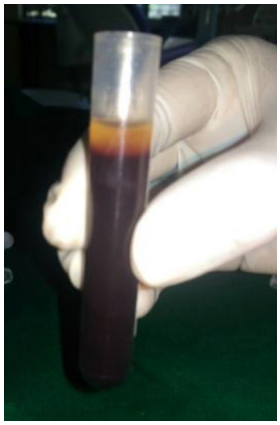

Figure-2: I-prf prepared

Figure-3: I-prf collected using insulin syringe

between baseline and 8 weeks were statistically significant (p≤0.05). The changes from baseline to 8 weeks represent an average of 51 % of increase in GB. At 6 weeks, 4 sites had ≥50 % increase in GB, while at 8 weeks the results improved and were maintained.

At first follow up (4 weeks after the injection), 15-20%

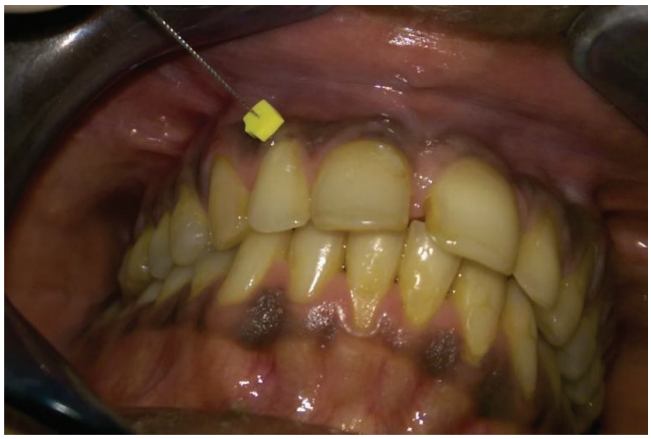

Figure-4: Transgingival recording of gingival thickness using endodontic file

Figure-5: Measurement recorded on digital vernier caliper

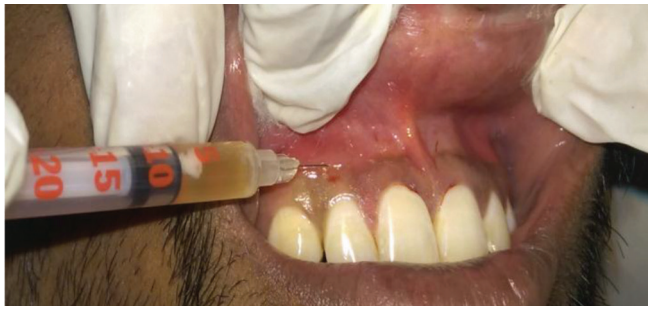

Figure-6: Injection of I-prf

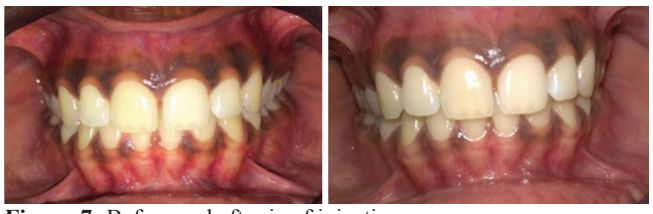

Figure-7: Before and after i-prf injections

GB enhancement with a mean of 0.79 ± 0.53 mm GT was observed (Table 2 and Figure 1). In the second follow up (6 weeks later) 25-30 % enhancement with a mean of 0.86 ± 0.05 mm GT and at the third follow up (8 weeks later), 50-60% enhancement with a mean of 1.025 ± 0.013 mm GT was noted. (Fig 7)

DICUSSION

Kao et al had suggested that the two tissue biotypes have different gingival and osseous architectures; hence they

exhibit different pathological responses when subjected to inflammatory, traumatic, or surgical insults. A thick gingival biotype is a requisite for good periodontal health as it can resist trauma and subsequent gingival recession, enable tissue manipulation and promote creeping attachment. This can be attributed to the high extracellular matrix and collagen volume, more layers of keratinisation, thick bony architecture which can withstand collapse, contraction and inflammation of thicker gingival tissue. On the other hand, a thin biotype is delicate, translucent, friable with a minimum zone of attached gingiva, thin bony architecture with possible presence of fenestrations and dehiscences, thereby is more prone to inflammation and trauma. Patients with thin scalloped biotypes are considered at risk as they have been associated with a compromised soft tissue response following surgical or restorative treatment.[14]

Hence a paradigm shift in periodontal treatment planning was inevitable wherein gingival tissue biotype was given due importance in order to develop more appropriate strategies for periodontal management which could result in more predictable treatment outcomes. The success of aesthetic reconstructive surgeries showed marked correlation with the gingival morphologic entities or biotypes at the surgical sites. Anderegg et al tried to evaluate whether the thickness of tissue used to cover guided tissue membrane influenced postsurgical recession, and he concluded that there was less post treatment gingival recession for tissue thickness >1mm than tissue thickness < or = 1mm.[15] Baldi et al in 1999 reported that a flap thickness of > 0.8 mm resulted in 100% root coverage, whereas a flap thickness of < 0.8 mm results in partial root coverage in CAF procedures of Miller's class I or II root coverage. He added that in coronally advanced flap, an average of 0.2 mm gingival recession decrease occurred for each 0.1 mm increase in flap thickness.[16] When relationship of dental implants with mucosal thickness was considered, Lee et al reported that mucosal thickness had a major influence on the degree of early peri-implant bone loss. [17]

To the best of author's knowledge, there are no studies evaluating a non-surgical approach for increasing keratinized tissue width (KTW) and GB in the current literature. The periodontal phenotype shows a stronger correlation with GB rather than KTW and papilla height. Therefore, GB was chosen as the main determinant parameter in this study. It was reported that ultrasound and transgingival probing yielded sufficient results for GB measurement. Henceforth, transgingival probing technique was preferred considering the cost, accuracy and reproducibility of this approach.

The initial PRF formulations lacked a liquid concentrate of proteins, as the standardized PRF membrane contains the majority of its growth factor concentration encapsulated within its fibrin matrix. For these reasons, major development and advancements were recently made with the aim of developing a liquid formulation of PRF which does not contain any anti-coagulants or fibrin matrix. These advancements were made possible due to the recent findings by Ghanaati et al. who introduced the low-speed concept for blood centrifugation whereby lower centrifugation speeds were shown to contain higher numbers of cells including leukocytes prior to the formation of a fibrin clot[9]. Leukocytes are immune cells having vast importance in tissue regeneration by directing and recruiting various cell types during the wound healing process. It was recently hypothesized that by reducing centrifugation G-force, a total increase in leukocyte numbers would remain in the top third layer of platelet concentrate tubes where PRP and PRF are collected. The added number of cells contained within this fibrin matrix was further shown to release higher total growth factor release of PDGF, TGF-β1, VEGF, EGF, and IGF when compared to control L-PRF. VEGF is a potent angiogenic factor and PDGF acts as a chemoattractant for cells of mesenchymal origin including fibroblasts providing a role in periodontal regeneration. Fibrin acts as a scaffolding hydrogel for agglomeration of adherent cells at the site of tissue healing. Additionally, fibrin is a carrier of growth factors in a well-controlled release system that sustains proper bioactivity over the healing period. [11,10]. Studies revealed a high release rate of PDGF and VEGF for 8 hrs and 24 hrs respectively, followed by a gradual decrease with time. It is even hypothesized that even following 10 days; an additional release of growth factors could still be expected from i-PRF.

A systematic review by Verma UP et al demonstrated that PRF enhances the GB, showed greater stability during remodelling and enhances blood supply to the underlying structures thereby affirming PRF as a therapeutic regenerative biomaterial.[18] Ozsagir ZB et al. in a very recent study showed a statistically significant 44% increase in Gingival thickness post iPRF usage. Multiple sessions with iprf injections are generally required to obtain desired results. Three sessions of i-PRF injections were performed at 2 week intervals in this study; considering the current PRF literature wherein PRF resorption time was 7–11 days, and growth factor release from i-PRF occurred at 10 days. A statistically significant improvement in gingival thickness was observed at 8 weeks which can attributed to the positive effects on neoangiogenesis and neocollagenesis.[19,20]

Although the gingival thickness post i-PRF injections increased on an average from 0.68 ± 0.12 to 1.025 ± 0.013 i.e. 51% increase; but this change still resides to be in the subclinical level. However, any patient-related factors such as return to previous traumatic brushing habit will cause less damage to the new anatomical structure gained compared with the baseline, because a structure more resistant to trauma has been obtained. The increase in gingival thickness obtained by the use of i-PRF injections prior to the surgical procedure may improve the success of periodontal surgery in individuals with thin gingival biotypes. Alveolar bone dehiscences that can result from orthodontic movements enlarge susceptibility to gingival recession, especially in subjects with the thin gingival biotype. Adding this procedure in orthodontic treatments may reduce the risk of gingival recession in individuals with thin biotypes.

Therefore, a statistically significant increase in gingival

thickness with i-PRF application in thin GB variants portrays this technique to be a new promising non-invasive approach to increase the thickness of gingiva. Nevertheless, further validations to these findings are necessary with longitudinal studies of larger sample sizes and long term follow-ups, with records of patient reported outcomes measures (PROMs) and patient reported experience measures (PREMs).

CONCLUSION

Current study results shows that application of i-PRF injections may increase gingival biotype, without a surgical intervention. Further studies will be useful to clarify this new technique and the limitations.

REFERNCES

1. Ochsenbein C, Ross S. A reevaluation of osseous surgery. Dent Clin North Am. 1969;13:87-102.
2. Seibert JL, Lindhe J. Esthetics and periodontal therapy. In: Lindhe J, editor. Textbook of Clinical Periodontology. 2nd ed. Copenhangen, Denmark: Munksgaard; 1989. p. 477-514.
3. Claffey N, Shanley D. Relationship of gingival thickness and bleeding to loss of probing attachment in shallow sites following nonsurgical periodontal therapy. J Clin Periodontol. 1986;13:654-7.
4. Weisgold AS. Contours of the full crown restoration. Alpha Omegan 1977;70:77-89.
5. Claffey N, Shanley D. Relationship of gingival thickness and bleeding to loss of probing attachment in shallow sites following nonsurgical periodontal therapy. J Clin Periodontol 1986;13:654-7.
6. Kothiwale S, Rathore A, Panjwani V. Enhancing gingival biotype through chorion membrane with innovative step in periodontal pocket therapy. Cell Tissue Bank. 2016;17:33-8.
7. Shetty SS, Chatterjee A, Bose S. Bilateral multiple recession coverage with platelet-rich fibrin in comparison with amniotic membrane. J Indian Soc Periodontol 2014;18:102-6
8. Dayoub, Suleiman & Al-Qershi, Mueataz. The Role of Platelet-Rich Fibrin in Increasing the Gingival Thickness and Modifying the Thin Gingival Biotype: A Comparative Clinical Split Mouth Study. Dent Hyp 2019;10:34.
9. Ghanaati, S., Al-Maawi, S., Herrera-Vizcaino, C., Alves, G. G., Calasans-Maia, M. D., Sader, R., Kirkpatrick, C. J., Choukroun, J., Bonig, H., & Mourão, C. A Proof of the Low Speed Centrifugation Concept in Rodents: New Perspectives for In Vivo Research. Tissue engineering. Part C, Methods, 2018;24:659–670.
10. Miron, R. J., Fujioka-Kobayashi, M., Hernandez, M., Kandalam, U., Zhang, Y., Ghanaati, S., & Choukroun, J. Injectable platelet rich fibrin (i-PRF): opportunities in regenerative dentistry?. Clinical oral investigations, 2017;21:2619–2627.
11. Miron, R. J., Fujioka-Kobayashi, M., Bishara, M., Zhang, Y., Hernandez, M., & Choukroun, J. Platelet-Rich Fibrin and Soft Tissue Wound Healing: A Systematic Review. Tissue engineering. Part B, Reviews, 2017;23:83–99.
12. Loe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. Acta Odontol Scand 1963;21:533-51.
13. Silness J, Loe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condtion. Acta Odontol Scand 1964;22:121-35.
14. Kao RT, Pasquinelli K. Thick vs. thin gingival tissue: a key determinant in tissue response to disease and restorative treatment. J Calif Dent Assoc. 2002 ;30:521-6.
15. Anderegg CR, Metzler DG, Nicoll BK. Gingiva thickness in guided tissue regeneration and associated recession at facial furcation defects. J Periodontol 1995;66:397–402.
16. Baldi C, Pini-Prato G, Pagliaro U. et al. Coronally advanced flap procedure for root coverage. Is flap thickness a relevant predictor to achieve root coverage? A 19-case series. J Periodontol 1999;70:1077-1084.
17. Lee A, Fu JH, Wang HL. Soft tissue biotype affects implant success. Implant Dent. 2011;20:e38-47.
18. Verma UP, Yadav RK, Dixit M, Gupta A. Platelet-rich Fibrin: a paradigm in periodontal therapy—a systematic review. J Int Soc Prev Community Dent. 2017;7:227-33.
19. Ozsagir ZB, Saglam E, Sen Yilmaz B, Choukroun J, Tunali M. Injectable platelet-rich fibrin and microneedling for gingival augmentation in thin periodontal phenotype: A randomized controlled clinical trial. J Clin Periodontol. 2020;47:489-499.
20. PR377: A new method to enhancing gingival biotype: injectable-platelet rich fibrin (I-PRF). J Clin Periodontol 2018; 45: 247-247.

Source of Support: Nil; **Conflict of Interest:** None

Submitted: 26-07-2021; **Accepted:** 27-07-2021; **Published:** 30-08-2021