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Effect of non-surgical periodontal therapy on salivary and gingival crevicular fluid concentration of visfatin in periodontal health and disease

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ABSTRACT

Background and objective: Visfatin, a pleotropic mediator mostly produced by visceral fat, is crucial in controlling the immunological and defensive systems. It serves the roles of a cytokine, an enzyme involved in energy metabolism, and a growth factor. The objective of the present study was to assess the impact of non-surgical periodontal therapy (scaling and root planing) on visfatin concentrations in saliva and gingival crevicular fluid in individuals with Periodontitis (stage-II grade-A)

Materials and methods: 54 individuals were divided into Group A (Periodontally Healthy) and Group B1(Periodontitis baseline) based on periodontal parameters including plaque index (PI), gingival index (GI), probing pocket depth (PPD), clinical attachment level (CAL), and radiographic parameters. After NSPT (SRP), Group B1 patients were recalled after 4 weeks, constituting Group B2 (post NSPT group B1). At baseline and 4 weeks after non-surgical periodontal therapy (SRP), all clinical parameters, salivary and GCF samples were recorded. An ELISA kit was used to measure the levels of visfatin. Using the paired *t*-test, unpaired *t*-test, and Pearson's correlation coefficient, data were analysed using SPSS 15.

Results: After non-surgical periodontal treatment (SRP), the mean salivary and gingival crevicular fluid concentration of visfatin considerably decreased to a level comparable to periodontal health. In all groups, GCF visfatin concentration was higher than salivary concentration of visfatin. In periodontitis patients, visfatin concentration in GCF was 1.5 times higher than in saliva.

Conclusion: The results of this investigation suggest a direct correlation between salivary and gingival crevicular fluid visfatin concentration and periodontal tissue inflammation and disease activity.

1. Introduction

A chronic inflammatory condition called periodontitis is characterized by the gradual deterioration of the tissues that support teeth and is linked to dysbiotic plaque biofilm.[1] Although bacteria are the major etiologic factor of periodontal diseases, studies have proved that periodontitis is multifactorial in origin.[2] The development of this condition is significantly influenced by factors including smoking, diabetes, and heredity.[3]

In addition to the direct impact of bacteria on periodontal tissues, bacteria also can indirectly harm the periodontium. Bacterial virulence mechanisms expose cells and underlying tissues to bacterial agents, which starts several destructive processes. The release of inflammatory mediators from adipose tissue, including visfatin, adiponectin, resistin and leptin, together with cytokines like Tumour Necrosis Factor – α (TNF-α), Interleukin-6 (IL-6) and Monocyte Chemoattractant Protein-1 (MCP-1), occurs in response to microbial assault. These elements and cytokines are considered to be crucial in immune responses and inflammation.[4] These cytokines can work independently or together to trigger inflammatory reactions and catabolic processes, such as bone loss and the breakdown of collagen by Matrix Metalloproteinases (MMP).[5] Even while bacteria are the cause of inflammatory lesions, pro-inflammatory mediators also play a substantial role in the loss of connective tissue and the deterioration of the supporting alveolar bone.[6] Pre-B-cell colony-enhancing factor, a 52-kDa protein structure that is now known as visfatin, was first discovered by **Fukuhara et al.** in

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2005.[7] It is a pleotropic mediator that serves as a pro-inflammatory mediator, a growth factor, a cytokine, and an enzyme involved in energy metabolism. Visfatin is found in a wide range of white blood cells, including tissue-bound macrophages, which suggests that it is crucial for the control of immunological and defensive processes.[8] Given that it may be present in inflammatory cells and a variety of inflammatory situations, it can be regarded as an inflammatory adipokine. According to studies, the expression of visfatin rises in inflammatory diseases that are both acute and chronic, such as type II diabetes mellitus, sepsis, acute pulmonary disorders, psoriasis, and rheumatoid arthritis.[9] Visfatin secretion by macrophages increases in unstable atherosclerotic lesions, which suggests a potential function for visfatin in the instability of the atherosclerotic plaque. Vascular disorders are likely to be at risk due to high blood visfatin levels.[10–12] Visfatin secretion may be considerably increased by elevated levels of pro-inflammatory cytokines such as IL-1, IL-6, and TNF- α.[13] It has been demonstrated that extracellular visfatin increases the synthesis of cytokines, matrix-degrading enzymes, and chemokines.[14,15] Visfatin prevents neutrophils from going through the apoptosis process which leads to persistence of inflammation and tissue deterioration which results from prolonged presence of polymorphonuclear leukocytes (PMNL) in the environment.[16]

Periodontal ligament cells generate visfatin, which is controlled by microbial, inflammatory, and biomechanical signals.[17,18] According to cellular research *P. gingivalis* and *F. nucleatum* increases the expression of visfatin.[19] Increased visfatin concentration in periodontal tissue boosts the production of MMP-1 and Chemokine (C–C motif) Ligand 2 (CCL2) also referred to as Monocyte Chemoattractant Protein-1 (MCP-1). By destroying COL-1, which is the form of collagen, MMP-1 plays crucial part in modelling and rebuilding the extracellular matrix whereas CCL-2 has an impact on T-cell immunity.[20]

A mechanical and chemotherapeutic method is used in non-surgical periodontal therapy (NSPT) to reduce or remove the microbial biofilm, which is the main cause of gingivitis and periodontitis. Given that NSPT (SRP) is regarded as the gold standard of periodontal treatment,[21] the aim of the present study was to find out the impact of NSPT (SRP) on the concentration of visfatin in saliva and GCF of patients with generalized periodontitis (stage-II grade-A). Rationale behind choosing GCF and Saliva was that to the best of my knowledge none of the studies conducted yet has analysed GCF and saliva together both being oral markers.

2. Materials and methods

Sample size is calculated on the basis of variation in the Salivary Visfatin concentration using the formula: **(Charan** and **Biswas 2013)**[31]

$$n = \frac{(Z_\alpha + Z_\beta)^2(\sigma_1^2 + \sigma_2^2)}{d^2}$$

Where $\sigma_1 = 2.297$, The Pre Treat SD of Salivary Visfatin concentration.
$\sigma_2 = 7.257$, The Post Treat SD of Salivary Visfatin concentration
$d = \min(\sigma_1, \sigma_1)$, the difference considered to be clinically significant. (Ref. Abolfazil et al.).[27]
type I error $\alpha = 5$ % corresponding to 95 % confidence level
type II error $\beta = 20$ % for detecting results with 80 % power of study.
Loss to follow up = 10 %
So the required sample size
n = 27 each group.

Based on inclusion and exclusion criteria, a total of 54 patients (27 men and 27 women) between the age of 18–50 years, reporting to the Periodontology Department's Outpatient unit were enrolled (Non-probability sampling, for comparison of two specific study groups[Healthy and Periodontitis group]). Ethical approval was taken from ethical committee [ECR/262/Inst/UP/2013/RR-19]. The inclusion criteria were patients within the age group of 18–50 years, who were systemically healthy with BMI (BMI = weight/height^2) of 18–24.9 kg/m^2. Exclusion criteria included subjects with systemic diseases, Obese patients with BMI more than 25 kg/m^2, Pregnant and lactating women, Subjects who were smokers and tobacco chewers, Subjects undergoing medical therapy, Subjects who had undergone any periodontal therapy in the last 6 months.

All the participants received a thorough explanation of the protocol, and provided their written consent. Each participant received a full-mouth periodontal probing and charting procedure, and an orthopantomogram was obtained to record the data dichotomously (presence or absence) to distinguish and to stage and grade patients with periodontitis from others. Based on the plaque index (PI), gingival index (GI), probing pocket depth (PPD), clinical attachment level (CAL), and radiographic evidence of bone loss, patients were divided into two groups. Group A (periodontally healthy) included 27 individuals who had radiographs that showed no signs of bone loss along with a clinically healthy periodontium, a plaque index [PI] ≤ 1, GI = 0 (lack of clinical inflammation), PPD ≤ 3 mm, and CAL ≤ 3 mm. Group B-1 (periodontitis baseline) included 27 participants with radiographic evidence of bone loss, symptoms of clinical inflammation, a plaque index [PI] ≥ 2, GI ≥ 1, PPD ≥ 5 mm, and CAL ≥ 5 mm. Patients with periodontitis (group B-1) were given SRP therapy single sitting and called back after four weeks (as fully epithelialized gingival crevice and connective tissue repair occur within 21 days) later to constitute Group B-2 (post-SRP group).

2.1. Saliva collection

Saliva samples of each individual was obtained at baseline and after 4 weeks (follow-up) using ***Draining method.***[22] From each patient, unstimulated whole expectorated saliva (3 ml) was collected in morning session. They were instructed not to take any food-stuffs, beverages and not to smoke 1 hour prior to sampling and were supposed to rinse their mouth meticulously several times with distilled water before sampling. Participants were seated in an upright position and asked to swallow first, and then told to allow saliva to dribble between parted lips into sterile eppendorfs. Collected samples were then centrifuged at 1000×*g* for 20 min at 2–8 °C as per ELISA manufacturer protocol. Samples were aliquoted prior to freezing at −80 °C for further analysis.

2.2. GCF collection

In periodontitis groups, only one site per individual was chosen as a sampling site, but in the healthy group, multiple sites that were free of inflammation were sampled to guarantee the collection of an acceptable amount of GCF. Sites with clinical attachment levels of >5 mm with symptoms of inflammation, and radiographic confirmation of bone loss was chosen for sampling.

Brill's technique[23] was used for GCF sample collection. The subjects were called in the morning and made to sit comfortably on the dental chair. Scaling was done to prevent contamination by plaque and calculus one day prior to sample collection. On the day of collection the site was then dried with a gentle stream of compressed air. Absorbent cotton rolls were used to maintain isolation during GCF collection. 3–4 paper points (Meta absobent paper point, size #20, 6 % taper) were gently placed in gingival sulcus until mild resistance was felt and it was held in situ for 30 s. Paper points that were soaked in blood or saliva were discarded. After GCF collection, paper points were transferred in a sterilized microcentrifuge tube (Eppendorf tube) containing 500 μl of phosphate buffer solution at 4 °C. Immediately after the transfer, sample was kept in a thermo-insulated flask having frozen ice cubes and was immediately transported to the Department of Biochemistry where they were stored at −80 °C until essayed; to keep the loss of reactivity of biomarkers to a minimum. Collected samples were then centrifuged at 1000×*g* for 20 min at 2–8 °C as per ELISA manufacturer protocol. Prepared GCF supernatant samples were evaluated for visfatin using ELISA kit.

2.3. Visfatin analysis

Using an ELISA kit that is available commercially, the samples were tested for the presence of human visfatin (Elabscience Biotechnology Inc. USA) in the Department of Biochemistry. The kit's minimum assay sensitivity was 0.19 ng/ml. Visfatin concentration was calculated in the appropriate wells in ng/ml.

2.4. Statistical analysis

Data was analysed using SPSS 15 statistical software. Paired *t*-test, unpaired *t*-test and pearson correlation test were also used for inter- and intra-group comparisons. Statistical significance was defined at $P < 0.05$.

2.5. Results

The descriptive statistics of the study including age and BMI are given in Table 1. Of 54 subjects in the present study, 27 were males and 27 females. There were no drop-outs. Paired *t*-test and unpaired *t*-test were used to compare clinical parameters and salivary and GCF visfatin concentration in healthy as well as in baseline (Group-B1) and follow-up patients (Group-B2). Results showed that clinical and biochemical parameters decreased after treatment (B1 Vs B2) as seen in Table 2 [Figure-1 and 2], which were statistically significant ($P < 0.001$). Pearson's correlation coefficient was used to correlate the relationship between salivary and gingival crevicular levels of visfatin (Table 3). The test showed a highly significant correlation between salivary and GCF levels of visfatin in all groups. Percentage raise in GCF visfatin with respect to salivary visfatin was estimated as seen in Table-4. 19.38 %, 143.84 % and 15.46 % raise was found in GCF visfatin values compared to salivary visfatin values in group A, B1 and B2 respectively.

3. Discussion

In this study, a total of 54 subjects (27 periodontally healthy and 27 periodontitis) were enrolled and baseline and post-treatment (4 weeks) assessment of clinical and biochemical markers were made following NSPT (SRP).

When compared to controls in our study, individuals with periodontitis had higher amounts of salivary and gingival crevicular fluid visfatin. When compared to the healthy controls (11.05 ng/ml), individuals with periodontitis had salivary visfatin levels that were considerably higher (29.47 ng/ml). This elevated level of visfatin might be the consequence of active secretion/transport from salivary glands, or this leakage could be caused by edema or cell membrane breakdown in periodontitis, or both. Additionally, visfatin levels in GCF were greater than in controls, 71.85 ng/ml and 13.19 ng/ml respectively [Table-2, -2].

Our findings are consistent with those of a research by **Tabari et al. (2014)**,[29] who reported that salivary visfatin levels were higher in those with chronic periodontitis than in healthy controls. Additionally, **Ozcan et al. (2014)**[24] discovered that individuals with gingivitis and periodontitis had greater salivary visfatin levels than healthy controls. This is also consistent with the findings of **Turer et al. (2016)**,[25] who found that individuals with chronic periodontitis had higher gingival crevicular visfatin levels than in patients with gingivitis or healthy controls. The research by **Pradeep et al. (2011),**[26] demonstarted that the concentration of visfatin in both the GCF and serum was lowest in healthy controls and highest in patients with gingivitis and periodontitis also supported the results of the present study.

Table-1
Descriptive statistics (mean ± SD) of the study population.

Groups	Healthy(A)	Periodontitis (B)	Overall
Age (years)	32.74 ± 7.30	36.52 ± 7.39	34.63 ± 7.52
BMI (kg/m^2)	22.43 ± 1.50	23.66 ± 2.94	23.05 ± 2.22

In our study, the decrease in visfatin levels in the saliva and gingival crevicular fluid following NSPT (SRP) was linked with the reduction of clinical parameters such as plaque index, gingival index, probing pocket depth, and clinical attachment level [Table-2]. In addition, it showed a direct relationship between periodontal tissue status and salivary and GCF levels of visfatin. Clinical parameters such as PI, GI, PPD, and CAL in the study by **Abolfazli et al. (2015)**[27] revealed a strong connection with serum and salivary levels of visfatin, which showed a decrease after treatment. **Pradeep et al. (2011)**[26] reported a weak and negative association between GCF visfatin concentrations and PPD in the healthy group, although it was not statistically significant. Similar results were reported for the weak but positive relationships between GCF visfatin concentrations and PPDs as well as GCF and CALs in chronic periodontitis patients, which were not statistically significant.

Our finding demonstrated that the decrease in gingival crevicular visfatin levels, which occurred four weeks after periodontal treatment from 71.85 ng/ml to 14.43 ng/ml, was statistically significant [Table-2, Figure-2]. Similar results were seen in study conducted by **Raghavendra et al. (2012)**[28] to evaluate the response of serum and GCF visfatin following NSPT, and the results showed that serum and GCF visfatin significantly reduced 2 months after NSPT. In our research salivary visfatin level dramatically decreased after NSPT from 29.47 ng/ml to 12.50 ng/ml in periodontitis patients [Table-2, Figure-2]. Our results were in agreement with a study by **Tabari et al. (2015)**[29] which demonstrated that salivary visfatin levels drastically reduced after NSPT. The drop in levels of visfatin following periodontal therapy may be related to the fact that the treatment reduced inflammation and the cytokines that mediate it, including IL-1 and IL-6, which together with visfatin contribute to chronic periodontitis.

In our study, controls and patients with periodontitis both had detectable levels of visfatin in their saliva and GCF. Patients with periodontitis had higher levels of visfatin in both saliva and GCF. Additionally, our study showed that NSPT causes individuals with periodontitis to have statistically significant lower salivary and gingival crevicular levels of visfatin, which also correspond with clinical parameters like PI, GI, PPD and CAL [Table-2, Figs. 1 and 2]. Also that there was a less significant difference in the levels of visfatin in both saliva and GCF of controls and post NSPT group, (0.004 and 0.006 respectively) depicting that NSPT reduces the level of visfatin to a nearly healthy level [Table-2, Figure-2]. **Anudeep Mopidevi et al. research's (2019)**,[30] which demonstrated that salivary levels of visfatin are lowered following periodontal therapy to levels equivalent with those reported in healthy persons, provided confirmation to our findings.

As compared to saliva, GCF visfatin concentrations were higher in all groups. GCF visfatin concentration increased in the healthy group (Group A) by 19.38 % and in the post-treatment group (Group B2) by 15.46 % in relation to saliva. A drastic increase in GCF visfatin concentration compared to salivary concentration was seen in periodontitis group (Group B1) i.e. 143.84 % [Table-4]. Visfatin as discussed above is present in detectable concentration in both saliva and GCF in healthy as well as in periodontitis patients, but its concentration as found in present study is higher in GCF. So GCF can be used to detect even small changes of visfatin concentration and helps in better estimation of periodontal condition. As seen in present study a highly significant correlation was observed between salivary and GCF levels of visfatin in Group A-B1, A-B2 and B1–B2 i.e. <0.001 [Table-3]. According to research done by **Abolfazli et al. (2015),**[27] no correlation was seen between the salivary and serum levels of visfatin.

An important difference between the present study and other studies was a difference in one of the samples i.e. none of the studies till now had compared both salivary and GCF samples for visfatin levels. This study also had a few limitations. First, the sample size was smaller. Second, the duration of the study was of short period of 4 weeks.

Various studies as previously mentioned, have shown that visfatin

Table-2
Comparison of visfatin concentration with clinical parameters.

Parameters	Group A	Group B1	Group B2	A vs B1		A vs B2		B1 vs B2	
				t-value	p-value	t-value	p-value	t-value	p-value
PI	0.34 ± 0.08	2.02 ± 0.31	0.48 ± 0.27	−27.4	< **0.001***	−2.72	**0.009***	18.53	< **0.001***
GI	0.20 ± 0.12	2 ± 0.23	0.39 ± 0.33	−36.79	< **0.001***	−2.79	**0.007***	18.66	< **0.001***
PPD	2.19 ± 0.30	4.54 ± 0.32	2.65 ± 0.78	−27.72	< **0.001***	−2.85	**0.006***	14.23	< **0.001***
CAL	2.21 ± 0.28	4.75 ± 0.43	2.70 ± 0.80	−25.55	< **0.001***	−2.68	**0.010***	13.30	< **0.001***
SALIVARY VISFATIN (ng/ml)	11.05 ± 1.51	29.47 ± 4.04	12.50 ± 1.98	−22.16	< **0.001***	−3.02	**0.004***	27.87	< **0.001***
GCF VISFATIN (ng/ml)	13.19 ± 1.67	71.85 ± 4.99	14.43 ± 1.54	−57.96	< **0.001***	−2.85	**0.006***	67.49	< **0.001***

*Statistically significant at $p < 0.05$. PI: Plaque Index. GI: Gingival Index. PPD: Probing Pocket Depth. CAL: Clinical Attachment Level.

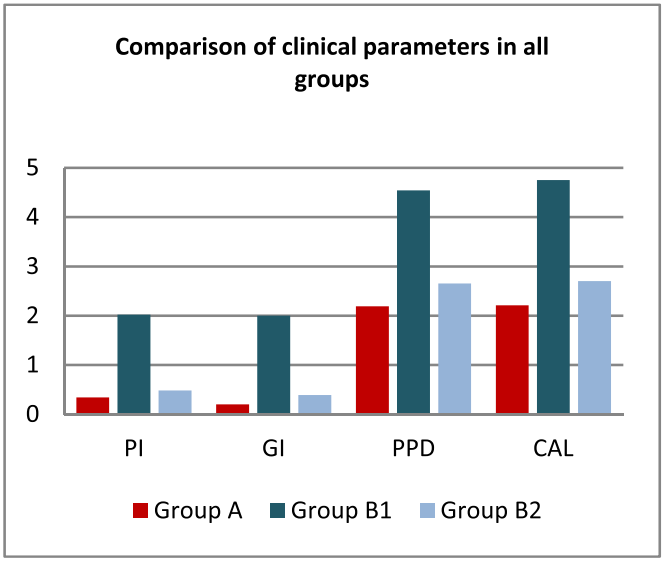

Figure-1. Comparison of clinical parameters in all groups.

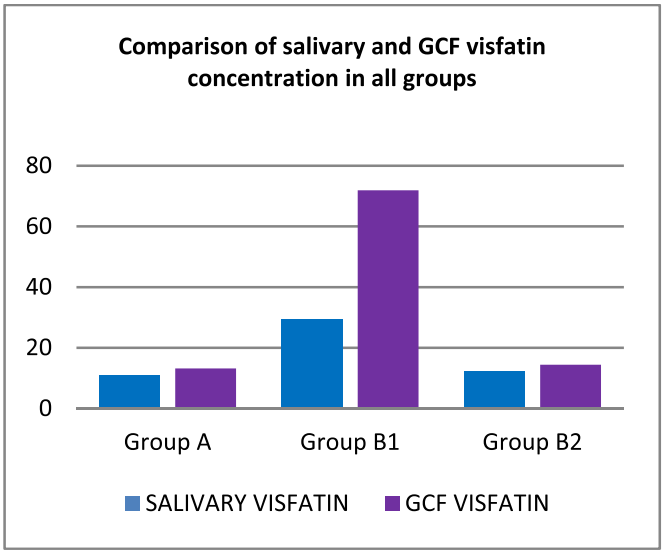

Figure-2. Comparison of salivary and GCF visfatin concentration in all groups. *Group A- Healthy subjects. Group B1- Baseline Periodontitis patients. Group B2-Follow-up Periodontitis patients. PI: Plaque Index. GI: Gingival Index. PPD: Probing Pocket Depth. CAL: Clinical Attachment Level.

levels rise in acute and chronic systemic inflammatory conditions as well as in chronic periodontitis. The advancement of periodontal disease may be a key factor in identifying people at high risk for today's most

Table 3
Pearson's correlation test for the salivary and GCF Visfatin concentration.

	GCF – Group A		GCF - Group B1		GCF – Group B2	
	r-value	p-value	r-value	p-value	r-value	p-value
Saliva (Group A)	0.808	< **0.001***	–	–	–	–
Saliva (Group B1)	–	-	0.737	< **0.001***	–	–
Saliva (Group B2)	–	-	–	-	0.818	< **0.001***

*Statistically significant at $p < 0.05$.

Table 4
Comparison between Salivary Visfatin and GCF Visfatin levels.

Groups	Parameters	Level		% raise in GCF wrt Saliva	paired *t*-test	
		Mean	SD		t-value	p-value
Gr A	Saliva (ng/ml)	11.05	1.51	19.38	−11.16	< **0.001***
	GCF(ng/ml)	13.19	1.67			
Gr B1	Saliva (ng/ml)	29.47	4.04	143.84	−64.91	< **0.001***
	GCF (ng/ml)	71.85	4.99			
Gr B2	Saliva (ng/ml)	12.50	1.98	15.46	−8.80	< **0.001***
	GCF(ng/ml)	14.43	1.54			

prevalent diseases, including diabetes, cardiovascular diseases, and others. Because periodontal therapy can lower visfatin levels, it's possible that it will also lower the chance of developing these disorders. Visfatin can also be used as a target to check the efficacy of a treatment for periodontal disease.

4. Conclusion

The following major conclusions were drawn from this study.

1 Patients with periodontitis had higher levels of salivary and gingival crevicular fluid visfatin than those people with healthy periodontium.
2. The clinical indicators such as plaque index, gingival index, probing pocket depth, and clinical attachment levels were reduced together with the levels of visfatin in the saliva and gingival crevicular fluid after NSPT (SRP) towards health.
3. The GCF concentration of visfatin was found to be significantly higher as compared to salivary visfatin concentration in all study groups and its concentration was approximately 1.5 folds higher in GCF of periodontitis patients.

Clinical significance of the study is that visfatin offers considerable promise as an early marker to detect disease activity and as a possible

therapeutic target for evaluating the efficacy of treatment due to the rising levels of salivary and gingival crevicular fluid visfatin in periodontitis and the subsequent lowering of this level by non-surgical periodontal therapy. Visfatin may be the missing link connecting periodontal illnesses and systemic diseases because of its close relationship with systemic diseases. In otherwise systemically healthy people, the increase in visfatin levels in periodontitis may exacerbate or cause such diseases.[8–10,12] Additionally, periodontal therapy with a lower level of this cytokine may lower the risk for such disorders. To validate the findings of our investigation, longer-term follow-up studies with a larger sample size are needed.

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Disclosure

None.

Ethical approval

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Declaration of competing interest

None to declare.

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