mRNA Expression of Ezrin in Gingival Crevicular Fluid and Whole Blood of Gingivitis and Chronic Periodontitis Patients – A Polymerase Chain Reaction Study

Abstract

Background: A comparative analysis of protein expression of gingival crevicular fluid (GCF) obtained from healthy individuals and individuals with periodontal diseases would help to identify proteins involved in periodontal disease progression. Among the identified proteins, Moesin which is a disease-associated protein belongs to the ezrin-radixin-moesin protein family and was proved to play an important role in the recognition of oral bacteria contributing to the consequent development of inflammatory immune responses involved in periodontal disease development. Aim: The aim of the study is to quantify and compare mRNA expression levels of ezrin in GCF and whole blood of gingivitis and chronic periodontitis patients. Materials and Methods: A total of 60 patients were selected for the study and were divided into three groups as follows: Group 1 (20 participants with healthy gingiva), Group 2 (20 participants with gingivitis), and Group 3 (20 participants with chronic periodontitis). Clinical parameters such as gingival index, periodontal index, probing pocket depth, and clinical attachment level were assessed. GCF and blood samples were taken from these patients and assessed for the mRNA expression of ezrin using real-time polymerase chain reaction. Results: The expression and mean relative quantification of mRNA expression of ezrin in GCF and blood were higher for periodontitis (18.32 ± 8.398, 19.34 ± 9.487) when compared to that of gingivitis (5.34 ± 3.609, 5.48 ± 4.428) and healthy individuals (2.33 ± 0.643, 3.47 ± 1.923) and they positively correlated with the clinical parameters. Conclusion: The increased expression of ezrin can be considered as a good indicator to assess the inflammatory activity in periodontitis and gingivitis.

Keywords: Blood, ezrin, gingival crevicular fluid, periodontitis

Introduction

The inflammation of the tooth-supporting periodontium could be considered as the initiation of a sequela of events, that would ultimately cause its irreversible damage. Numerous proteins and biochemical markers have been studied to better understand the cause and progression of periodontal inflammation. The ezrin-radixin-moesin (ERM) family consisting of ERM is expressed in several epithelial and mesothelial cells. The presence of ezrin in the plasma membrane of neutrophils and its potent role in inflammation have been described in the literature. Hence, an attempt has been made in the study to quantify and compare mRNA expression levels of ezrin in gingival crevicular fluid (GCF) and whole blood of gingivitis and chronic periodontitis to determine if ezrin could be considered as a biomarker for periodontal disease, as limited studies are currently available in the literature that has explored the association between ezrin and periodontitis.

Materials and Methods

Study design

Patients were randomly selected from the outpatient section of the Department of Periodontology of the institution. The study was approved by the Institutional Ethical Committee and was according to the Declaration of Helsinki 1975, as revised in 2000. This randomized case–control study was registered under the Clinical Trials Registry, India (CTRI/2018/04/013457) and was carried out for 9 months from January 2017 to September 2017. All the participants were explained about the need and design of the study, and written informed consent was obtained from each patient. The patients selected for the study

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underwent a full mouth periodontal probing and charting and were screened for their eligibility for the study.

**Inclusion criteria and clinical data**

The following patients have been included in this study as follows: (i) patients diagnosed with chronic periodontitis (5 or more teeth with probing depth ≥5 mm and clinical attachment loss ≥3 mm and gingival index ≥2), (ii) patients who were systemically healthy with probing depth ≤3 mm or no clinical attachment loss and gingival index score <1 and who have not received periodontal treatment for at least 6 months before the clinical examination and sampling, (iii) patients with gingivitis (probing depth ≤3 mm or no clinical attachment loss and gingival index ≥1 and ≤2), and (iv) all participants willing to participate in the study. The following patients were excluded from the study as follows: (i) smokers, (ii) patients with systemic diseases such as diabetes mellitus, heart diseases, rheumatoid arthritis, hepatitis, and other systemic diseases that can alter the course of periodontal disease, (iii) participants on any medication taken within the past 6 months which may alter the periodontal status, (iv) pregnant and lactating mothers, and (v) patients who have received any periodontal therapy in the past 6 months.

A case history proforma was designed to have a systematic and methodical recording of all observations and information. The relevant data were recorded which included clinical parameters such as gingival index (Loe and Silness, 1963), periodontal index (Russell, 1956), probing pocket depth (PPD), and clinical attachment level (CAL). PPD was measured using a graduated Williams periodontal probe from the crest of the gingival margin to the base of the pocket, and CAL was measured from cementoenamel junction to the base of the pocket.

A total of 120 samples were collected from 60 patients. 120 samples were divided as follows:
- Group 1: 20 GCF samples and 20 whole blood samples from healthy individuals
- Group 2: 20 GCF samples and 20 whole blood samples from gingivitis patients
- Group 3: 20 GCF samples and 20 whole blood samples from chronic periodontitis patients.

GCF and whole blood samples were collected from each participant of all the three groups for analysis of mRNA expression of Ezrin.

**Sampling and analysis**

GCF was collected by drying the gingival surface with sterile cotton, after which the region was isolated to stop contamination with saliva. In the healthy group, to ensure an adequate volume, GCF was pooled from multiple sites with no inflammatory signs and gingival index score <1. In gingivitis patients, the site with the highest gingival index score in the absence of CAL was selected. In chronic periodontitis patients, sites with ≥3 mm CAL were identified using a Williams graduated periodontal probe, and sites showing the highest gingival index score and highest CAL and deepest PPD were selected for sampling. On the sample collection day, after gently drying the area with a blast of air, the supragingival plaque was removed from the interproximal surfaces with a sterile curette. A graduated micropipette (Sigma Aldrich, St Louis, MO, USA) was kept at the entrance to avoid any blood contamination which could occur if the micropipette was inserted subgingivally. From each test site, an attempt was made to collect a standardized volume of 3 µl using the calibration on the microcapillary pipette with an extracrevicular approach (without stimulating the gingiva). In case of visible blood contamination, the sample was discarded and collected again. The GCF collected was pipetted using a blower, and the entire volume of collected GCF was transferred to Eppendorf tubes (0.5 ml), centrifuged at 3000 rpm for 10 min, and stored at −80°C until the time of assay (within a week).[6]

Four milliliters of venous blood was collected from the antecubital vein by a standard venepuncture method and stored in test tubes coated with EDTA at −80°C until analysis.[6] mRNA expression of ezrin in GCF and whole blood samples were analyzed using real-time polymerase chain reaction technique (RT-PCR) using Custom TaqMan® gene expression Assay (Applied Biosystems, India) for ezrin as per the manufacturer’s instructions.

**Statistical analysis**

The results for each parameter averaged (mean ± standard deviation) are presented in tables. Shapiro–Wilk test (P < 0.05) and a visual inspection of their histograms, normal Q–Q plots, and box plots showed that the clinical parameters and ezrin levels in GCF and whole blood were normally distributed in study groups.

Data were statistically analyzed using the one-way ANOVA test for mean comparison between the groups, and Tukey’s multiple post hoc test was used for pair-wise comparison between the groups. Correlation between clinical parameters and ezrin levels in GCF and whole blood was assessed using Spearman’s rank correlation. Data were analyzed using SPSS version 18.5 for Windows (SPSS Inc., Chicago, IL, USA).

The sample size was determined based on power analysis at an alpha error of 5%, and the power of the study was determined as 90%. The effect size calculated was 1.017 using G power software and SPSS software.

**Results**

The present case–control study was contemplated to evaluate the expression of ezrin and quantify their expression in GCF and whole blood in healthy individuals, gingivitis, and chronic periodontitis patients using the
RT-PCR technique. We have compared the levels of mRNA expression of ezrin in GCF and whole blood and correlated them with clinical parameters such as gingival index, periodontal index, PPD, and CAL.

The mean age and gender distribution of participants in the three different groups are shown in Table 1. Comparison of mean gingival index scores and periodontal index scores for healthy controls, gingivitis patients, and chronic periodontitis patients was statistically significant \( (P < 0.001) \). The mean probing depth in the chronic periodontitis group was significantly higher when compared to other groups and was statistically significant \( (P < 0.001) \). Intergroup comparison between the groups showed that three clinical parameters (mean gingival index, mean periodontal index, and mean PPD) are statistically significant \( (P < 0.001) \) [Table 2].

The present study revealed that expression of ezrin in GCF was higher in chronic periodontitis patients (65%) when compared to that of gingivitis (50%) and healthy controls (15%) and their expressions were statistically significant \( (P = 0.005) \). Similarly, the expression of ezrin in whole blood was marginally higher in patients with chronic periodontitis (70%) when compared to that of gingivitis (60%) and healthy controls (55.5%) and their expressions were statistically not significant \( (P = 0.610) \) [Table 3].

### Comparison of mean mRNA expression of ezrin in gingival crevicular fluid

The mean relative quantification (RQ) of mRNA expression of ezrin in GCF was \( 2.33 \pm 0.643, 5.34 \pm 3.609, \) and \( 18.32 \pm 8.398 \), respectively, in healthy controls, gingivitis, and chronic periodontitis patients, and the difference in mean mRNA expressions was statistically significant \( (P = 0.004) \). Pairwise comparison of the three groups showed that the mean RQ of mRNA expression of ezrin in GCF was statistically significant between the healthy and chronic periodontitis group \( (P = 0.002) \) and gingivitis and chronic periodontitis group \( (P < 0.001) \) whereas between the healthy and gingivitis group, it was statistically not significant \( (P = 0.762) \) [Table 4].

### Comparison of mean mRNA expression of Ezrin in whole blood

The mean RQ of mRNA expression of ezrin in whole blood was \( 3.47 \pm 1.923, 5.48 \pm 4.428, \) and \( 19.34 \pm 9.487 \), respectively, in healthy controls, gingivitis, and chronic periodontitis patients, and the difference in mean mRNA expressions was statistically significant \( (P = 0.004) \). Pairwise comparison of the three groups showed that the mean RQ of mRNA expression of ezrin in whole blood was statistically significant between the healthy and chronic periodontitis group \( (P = 0.002) \) and gingivitis and chronic periodontitis group \( (P < 0.001) \) whereas between the healthy and gingivitis group, it was statistically not significant \( (P = 0.762) \) [Table 4].

### Table 1: Distribution of participants according to age and gender in the various study groups

| Age          | Gender | n   | Mean±SD | F      | P         | F                   | P         |
|--------------|--------|-----|---------|--------|-----------|---------------------|-----------|
|              | Male, n (%) | Female, n (%) |        |        |           |                     |           |
| Healthy      | 20     | 25.1±4.097 | 25.390 | <0.001 | 10 (50)   | 10 (50)             |           |
| Gingivitis   | 20     | 28.8±4.137 | 10 (50) | 10 (50) |           |                     |           |
| Periodontitis| 20     | 36.8±7.157 | 10 (50) | 10 (50) |           |                     |           |

\( P < 0.05 \) is statistically significant. SD: Standard deviation.

### Table 2: Comparison of three groups with gingival index, periodontal index, probing pocket depth, and clinical attachment level

| Comparison of clinical parameters using one-way ANOVA | n   | Mean±SD | F      | P         |
|-----------------------------------------------------|-----|---------|--------|-----------|
| GI                                                   |     |         |        |           |
| Healthy (1)                                          | 20  | 0.14±0.067 | 978.041 | <0.001*   |
| Gingivitis (2)                                       | 20  | 1.44±0.166 |         |           |
| Periodontitis (3)                                    | 20  | 2.19±0.184 |         |           |
| PI                                                   |     |         |        |           |
| Healthy (1)                                          | 20  | 0.27±0.078 | 555.851 | <0.001*   |
| Gingivitis (2)                                       | 20  | 1.52±0.214 |         |           |
| Periodontitis (3)                                    | 20  | 3.43±0.469 |         |           |
| PPD                                                  |     |         |        |           |
| Healthy (1)                                          | 20  | 1.57±0.396 | 317.660 | <0.001*   |
| Gingivitis (2)                                       | 20  | 2.01±0.401 |         |           |
| Periodontitis (3)                                    | 20  | 7.15±1.226 |         |           |
| CAL                                                  |     |         |        |           |
| Periodontitis (3)                                    | 20  | 5.10±1.119 |         |           |

Pairwise comparison between the groups using Tukey's test

| Mean difference | P         | Mean difference | P         | Mean difference | P         |
|-----------------|-----------|-----------------|-----------|-----------------|-----------|
| GI              |           |                 |           |                 |           |
| 1 versus 2      | −1.30     | <0.001          | −2.04     | <0.001          | −0.074    | <0.001   |
| 1 versus 3      | −1.24     | <0.001          | −3.15     | <0.001          | −1.91     | <0.001   |
| 2 versus 3      | −0.44     | 0.182           | −5.58     | <0.001          | −5.14     | <0.001   |

*P < 0.05 is statistically significant. SD: Standard deviation; GI: Gingival index; PI: Periodontal index; PPD: Probing pocket depth; CAL: Clinical attachment level.
periodontitis patients, and the difference in mean mRNA expressions was statistically significant ($P < 0.001$). Pairwise comparison of the three groups showed that the mean RQ of mRNA expression of ezrin in whole blood was statistically significant between the healthy and chronic periodontitis group ($P < 0.001$) and gingivitis and chronic periodontitis group ($P < 0.001$) whereas between healthy and gingivitis group, it was statistically not significant ($P = 0.739$) [Table 5].

**Correlation of mRNA expression of ezrin in gingival crevicular fluid, blood with clinical parameters**

In healthy controls, mRNA expression of ezrin in GCF positively correlated with mRNA expression of ezrin in blood ($r = 0.979$) and PPD ($r = 0.996$). mRNA expression of ezrin in whole blood positively correlated with the PPD ($r = 0.529$), gingival index ($r = 0.029$), and periodontal index ($r = 0.150$).

In gingivitis patients, mRNA expression of ezrin in GCF positively correlated with mRNA expression of ezrin in blood ($r = 0.969$), gingival index ($r = 0.125$), periodontal index ($r = 0.231$), and PPD ($r = 0.214$). mRNA expression of ezrin in whole blood positively correlated with gingival index ($r = 0.241$), periodontal index ($r = 0.465$), and PPD ($r = 0.461$).

In chronic periodontitis patients, mRNA expression of ezrin in GCF positively correlated with mRNA expression of ezrin in blood ($r = 0.961$), periodontal index ($r = 0.539$), PPD ($r = 0.911$), and CAL ($r = 0.867$). mRNA expression of ezrin in whole blood positively correlated with gingival index ($r = 0.359$), periodontal index ($r = 0.553$), PPD ($r = 0.792$), and CAL ($r = 0.584$) [Table 6].

**Discussion**

Unresolved inflammation and fibrosis progressing to loss of tissue function are a few hallmarks of chronic and aggressive periodontal disease. An antibody response is mounted as the process of inflammation recruits a variety of cells of adaptive immunity. Neutrophils express many integrins associated with the process of transendothelial migration. Inflammation facilitates the interaction between CD11b/CD18 on neutrophils and intercellular cell adhesion molecule-1 (ICAM-1) on endothelial cells and C3bi opsonized pathogens, which promotes calcium release aided by tyrosine kinase. Calcium ($Ca^{2+}$) signaling is vital for the propagation of response to extracellular stimuli. The possible mediator of calcium signaling in neutrophils is the enzyme calpain. The migration of neutrophils ensues as calpain gets activated on sensing a high concentration of cytosolic $Ca^{2+}$.

The role of calpain in enabling the neutrophil to adhere to endothelial cells during transendothelial migration and in the process of phagocytosis is predominant. $\mu$Calpain in neutrophils which is associated with cytoskeletal reorganization cleaves ezrin of the ERM family but not moesin. This displays a

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**Table 3: mRNA expression of ezrin in gingival crevicular fluid and whole blood in all the three groups**

| Ezrin in GCF         | Healthy (n=20), n (%) | Gingivitis (n=20), n (%) | Periodontitis (n=20), n (%) | P    |
|----------------------|-----------------------|--------------------------|-----------------------------|------|
| Ezrin in GCF         | 3 (15.0)              | 10 (50.0)                | 13 (65.0)                   | 0.005|
| Ezrin in whole blood | 11 (55.5)             | 12 (60.0)                | 14 (70.0)                   | 0.610|

$P<0.05$ is statistically significant. GCF: Gingival crevicular fluid

**Table 4: Comparison of mean mRNA expression of ezrin in gingival crevicular fluid**

Comparison of mean mRNA expression of ezrin in GCF using one-way ANOVA

| Ezrin in GCF | n     | Mean±SD     | F      | P    |
|--------------|-------|-------------|--------|------|
| Healthy (1)  | 3     | 2.33±0.643  | 14.728 | 0.004|
| Gingivitis (2)| 10    | 5.34±3.609  |        |      |
| Periodontitis (3) | 13    | 18.32±8.398 |        |      |

Pairwise comparison of mean mRNA expression of ezrin in GCF using Tukey’s test

| 1 versus 2 | Mean difference | P       | 1 versus 3 | Mean difference | P       | 2 versus 3 | Mean difference | P       |
|------------|-----------------|---------|------------|-----------------|---------|------------|-----------------|---------|
| −3.01      | 0.762           | −15.98  | 0.002      | −12.97          | <0.001  |

$P<0.05$ is statistically significant. GCF: Gingival crevicular fluid; SD: Standard deviation

**Table 5: Comparison of mean mRNA expression of ezrin in whole blood**

Comparison of mean mRNA expression of ezrin in whole blood using one-way ANOVA

| Ezrin in whole blood | n     | Mean±SD     | F*     | P    |
|----------------------|-------|-------------|--------|------|
| Healthy (1)          | 11    | 3.47±1.923  | 23.120 | <0.001|
| Gingivitis (2)       | 12    | 5.48±4.428  |        |      |
| Periodontitis (3)    | 14    | 19.34±9.487 |        |      |

Pairwise comparison of mean mRNA expression of ezrin in whole blood using Tukey’s Test

| 1 versus 2 | Mean difference | P       | 1 versus 3 | Mean difference | P       | 2 versus 3 | Mean difference | P       |
|------------|-----------------|---------|------------|-----------------|---------|------------|-----------------|---------|
| −2.01      | 0.739           | −15.87  | <0.001     | −13.86          | <0.001  |

$P<0.05$ is statistically significant. SD: Standard deviation. F value close to 1 means null hypothesis is true. A larger F value signifies large variations among group means.

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Table 6: Correlation between mRNA expression of ezrin in gingival crevicular fluid, blood, and all clinical parameters among the three groups

| Group       | Ezrin in blood | GI    | PI    | PPD  | CAL  |
|-------------|----------------|-------|-------|------|------|
| Healthy     | Ezrin in GCF   |       |       |      |      |
| Correlation | 0.979          | −1.000| −0.726| 0.996|      |
| P           | 0.130          | 0.010 | 0.483 | 0.060|      |
| n           | 3              | 3     | 3     | 3    |      |
| Ezrin in blood |              |       |       |      |      |
| Correlation | 0.029          | 0.150 | 0.529 |      |      |
| P           | 0.932          | 0.660 | 0.094 |      |      |
| n           | 11             | 11    | 11    |      |      |
| Gingivitis  | Ezrin in GCF   |       |       |      |      |
| Correlation | 0.969          | 0.125 | 0.231 | 0.214|      |
| P           | <0.001*        | 0.732 | 0.521 | 0.552|      |
| n           | 9              | 10    | 10    | 10   |      |
| Ezrin in blood |              |       |       |      |      |
| Correlation | 0.241          | 0.465 | 0.461 |      |      |
| P           | 0.450          | 0.128 | 0.132 |      |      |
| n           | 12             | 12    | 12    |      |      |
| Periodontitis | Ezrin in GCF  |       |       |      |      |
| Correlation | 0.961          | −0.071| 0.539 | 0.911| 0.867|
| P           | 0.000          | 0.818 | 0.057 | <0.001| <0.001|
| n           | 11             | 13    | 13    | 13   | 13   |
| Ezrin in blood |              |       |       |      |      |
| Correlation | 0.359          | 0.553 | 0.792 | 0.584|      |
| P           | 0.207          | 0.040 | 0.001 | 0.028|      |
| n           | 14             | 14    | 14    | 14   |      |

P<0.05 is statistically significant. GI: Gingival index; PI: Periodontal index; PPD: Probing pocket depth; CAL: Clinical attachment level; GCF: Gingival crevicular fluid

level of selectivity for ezrin as a substrate, thus proving its role in inflammation.

We found that the expression of Ezrin in GCF is significantly higher in chronic periodontitis patients when compared to that of gingivitis and healthy individuals and the expression of Ezrin in whole Blood is marginally higher in patients with chronic periodontitis when compared to that of the other two groups with statistically no difference in their expressions between groups [Figures 1 and 2]. Shcherbina et al. determined the levels of ezrin in blood cells and found it to vary from as low as <0.1 µ/mg cell protein in erythrocytes and megakaryocytes to 0.5, 0.7, and 1.3 µ/mg protein, respectively, in neutrophils, monocytes, and lymphocytes.[12] Hence, the presence of ezrin in the blood is a normal finding, as could be attributed to this study which found varied levels of ezrin among the three groups considered. In the present study, the mean RQ of mRNA expression of ezrin is found to be higher in chronic periodontitis (19.34 ± 9.487) when compared to that of the gingivitis group (5.48 ± 4.428) and healthy group (3.47 ± 1.923) which can be explained by the fact that a higher level of local and systemic inflammation influences the inflammatory reaction in the gingiva. Andriankaja et al. found that there was an increased presence of local and systemic inflammatory mediators associated with gingivitis.[13]

Siqueira et al. reported that 17.8% of the pellicle proteins are derived from GCF.[14] Ezrin was found in the pellicle of all the patients in the study conducted by Delius et al.[15] and a part of this ezrin might be derived from the GCF as a major amount of the protein forming the pellicle comes from the GCF.

In this present study, we have found quantitatively increased levels of Ezrin expression in the GCF of patients with periodontitis followed by the gingivitis group when compared to that of healthy individuals [Figures 3 and 4]. Thus, it can be stated that the expression of ezrin increases with the increase in extent and severity of the periodontal disease. The more the severity, the more is the level of inflammation, leading to more expression of ezrin. As periodontitis and gingivitis, both are inflammatory conditions and both stimulate chemokines, which may lead to increased expression of Ezrin.

Leslie et al. conducted a study to assess the increased expression of ezrin-binding phosphoprotein (EBP50) during vascular remodeling.[16] This protein leads to increased macrophage activation and the enhanced response of vascular cells to inflammation. During inflammation, there is transient vessel dilation, followed by constriction. This vascular remodeling occurs during gingivitis and periodontitis as they are inflammatory conditions, and this vascular remodeling may lead to the increased ezrin expression in the GCF as found in our study. Kaczor-Urbanoowicz et al. found ezrin in 48 patients with orthodontically induced root resorption in the whole saliva of the patients.[17] These data reconfirm the association of ezrin to the inflammatory process.

Pore et al. found that conditional deletion of ezrin in B-cells increases B-cell interleukin-10 (IL-10) production.[18] Thus, ezrin activation due to inflammation in the B-cells of the GCF would not be able to produce IL-10, which is necessary for antibody formation. Thus, increased levels of ezrin in the GCF may be correlated as a poor prognostic indicator of periodontitis and gingivitis as increased levels of ezrin is consistent with persistent inflammation and hampered humoral immunity as supported by the studies. Hence, the role of ezrin in inflammation is understood by the above-mentioned studies.

Members of the ERM family recognize lipopolysaccharide (LPS) and lead to macrophage activation and play a role in innate immune response in periodontal diseases. LPS is a major component of the
outer membrane of Gram-negative bacteria contributing to the surface integrity of bacteria. The bacteria associated with periodontal diseases are predominantly Gram-negative bacteria. The increased expression of ezrin in the GCF, in patients with chronic periodontitis, may be directly related to the amount of bacterial load, as more the Gram-negative bacteria more would be the LPS, and more would be the expression of ezrin. However, more studies have to be made to establish a direct correlation between bacterial load and the expression of ezrin to prove the hypothesis.

Tsuchida et al. found ezrin in GCF from two patients with severe periodontal disease. This present study was conducted to evaluate and quantify the expression of ezrin in GCF and whole blood among healthy individuals, gingivitis, and chronic periodontitis patients. Based on the aforementioned findings, we can hypothesize that increased expression of ezrin in individuals can be considered as an indicator of the extent and severity of inflammation in periodontal tissues and can be considered as a diagnostic marker in periodontal diseases. To the best of our knowledge, this is the first study in this regard. Further studies conducted on a larger sample involving different perspectives of ezrin might lead to the exploration of the diagnostic potential of ezrin in periodontal and gingival diseases.

Limitations

1. The positive correlation between increased bacterial load and amount of ezrin has been hypothesized in our study but more specific study to relate the same is required
2. An increase in expression of inflammatory mediators in blood might lead to an increasing amount in GCF, as it is the ultrafiltrate of blood. The current study is, however, unable to establish a direct relationship, and hence, further research may be needed
3. The influence of ezrin as a prognostic marker has not been evaluated in our study as the primary focus was to determine the expression of ezrin in normal participants compared to those with gingivitis and periodontitis.
However, the findings of our study pave the path for consecutive studies to determine the prognostic significance of ezrin.

**Conclusion**

Within the limits of the present study, it can be concluded that the increased expression of ezrin can be considered as a good diagnostic indicator for inflammatory activity in periodontitis and gingivitis.

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**Conflicts of interest**

There are no conflicts of interest.

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