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Altered expression of SOCS genes in periodontitis

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Abstract

Suppressor of cytokine signalling (SOCS) family comprises a group of proteins that impede JAK/STAT signalling, thus being involved in the pathogenesis of immune-related conditions. In the present work, we aimed at identification of the role of SOCS genes in the pathogenesis of periodontitis through evaluation of their expression levels both in the circulation and in the affected tissues of patients. Thus, we measured expression levels of SOCS1-3 and SOCS5 transcripts in the blood and gingival samples of patients with periodontitis in comparison with control samples obtained during dental crown lengthening. Expressions of SOCS1, SOCS2, SOCS3 and SOCS5 genes were similar between gingival tissues of patients and controls. However, our results demonstrated under-expression of SOCS1 in blood samples of patients compared with controls (Ratio of mean expression (RME) = 0.47, *P* value = 0.04). The same pattern was observed among female subjects (RME = 0.38, *P* value = 0.04). SOCS2 was down-regulated in blood samples of female patients compared with female controls (RME = 0.22, *P* value = 0.04). SOCS3 was also under-expressed in the circulation of total cases versus total controls (RME = 0.29, *P* value = 0.02) and in female patients compared with female controls (RME = 0.19, *P* value = 0.04). Expression of SOCS5 was not different between blood samples two study groups. SOCS2 had the best function in separation of affected tissues from unaffected ones (AUC = 0.66, sensitivity = 0.39, specificity = 0.83). SOCS3 was superior to other transcripts in differentiation of blood samples of patients from normal blood samples (AUC = 0.69, sensitivity = 0.81, specificity = 0.68). Combination of transcript levels of SOCS1, SOCS2, SOCS3 and SOCS5 genes enhanced the AUC values to 0.64 and 0.67 in tissue and blood specimens, respectively. Taken together, certain SOCS genes have been found to be dysregulated in the circulation of patients with periodontitis.

Keywords: SOCS, JAK/STAT, Periodontitis

Introduction

Periodontal disorders impose an important burden on health [1]. The resident flora of the oral cavity contributes in the pathogenesis of periodontal disorders. Some central features of the oral microbiome have been clarified using metagenomic and 16S gene analyses [2]. These techniques have also facilitated establishment of

personalized medicine approaches that incorporates genomic and clinical data to forecast possible predisposition to these disorders [3]. Thus, expression assays in tissues obtained from patients with periodontitis have practical significance. Suppressor of cytokine signalling (SOCS) family comprises a group of molecules that inhibit JAK/STAT signals [4]. JAK/STAT signalling is a cytokine-induced pathway that regulates various critical biological responses, particularly immune function [4]. This family consists of eight proteins, namely CIS and SOCS1-SOCS7, all of them having a SH2 domain, SOCS box, and a distinctive N-terminal part [5]. SOCS1-3 and CIS are proteins whose expressions are stimulated

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by cytokines or growth factors, leading to suppression of JAK/STAT-associated cytokine signalling through a negative feedback circuit [4]. SOCS5 is another member of this family that contain SOCS box, yet the biological effects of this protein are not completely understood. Expression of this protein has been detected in primary B and T lymphoid cells, yet it does not exert essential function in production and activity of these cells [6].

Consistent with the important roles of SOCS proteins in the modulation of inflammatory responses, these proteins participate in the pathogenesis of related disorders. Periodontitis is among complex disorders in which inflammatory responses regulate disease progression [7]. A number of previous studies have indicated the role of SOCS proteins in this disorder. For instance, expression of SOCS1 has been found to be increased in the experimental model of periodontitis, parallel with activation of STAT1 and NF- κ B pathways [8]. Moreover, down-regulation of SOCS3 in myeloid cells has resulted in enhancement of *Porphyromonas gingivalis*-induced inflammatory responses and over-production of interleukins-1 β , -6, and -8 by peritoneal macrophages [9].

SOCS proteins have a crucial role in the regulation of microbial pathogen-associated signaling of cytokines, mainly via suppression of the activity of JAK/STAT cascade. In addition, they are involved in various processes exploited by bacteria to evade host defense [10].

SOCS1-3 have also been suggested to contribute in the regulation of bone resorption in the context of periodontitis [11]. Therefore, assessment of expression of these genes might facilitate identification of the underlying cause of periodontitis and pathogenic events associated this disorder.

In this work, we aimed at identification of the role of SOCS genes in the pathogenesis of periodontitis through evaluation of their expression levels both in the circulation and in the affected tissues of patients. Thus, we measured expression levels of *SOCS1-3* and *SOCS5* transcripts in the circulation and gingiva of patients with periodontitis in comparison with control samples obtained during dental crown lengthening.

Materials and methods

Enlisted persons

Whole blood samples were obtained from patients with periodontitis and control subjects. Gingival samples were obtained from individuals affected with chronic periodontitis, similar to the method explained in our former study [12]. Inclusion criteria were chronic periodontitis (Stage II to IV), probing depth of 5 mm or greater, bleeding on probing, and a minimum of 3 mm attachment loss requiring surgical periodontal management. We excluded

individuals who had history of smoking, systemic disorders, diabetes mellitus and consumption of antibiotics or anti-inflammatory agents. All patients were assessed in a university-affiliated periodontal clinic. Control samples were excised during dental crown lengthening procedure. Ethical committee of Shahid Beheshti University of Medical Sciences permitted the conduction of study based on the ethical guidelines (Ethical code IR.SBMU.DRC.REC.1400.012).

Assessment of gene expression

PicoPureTM RNA Isolation Kit (Applied Biosystems) was used for RNA extraction. Then, cDNA was produced from these samples by using the cDNA production kit (Yektatajhez, Iran). Expressions of *SOCS1-3* and *SOCS5* genes were measured in tissues and blood samples using the qRT-PCR kit (Ampliqon, Denmark) in LightCycler[®] 96 machine. Reactions were performed in duplicate. Details about PCR conditions and primers are reported in our former study [13].

Statistical methods

Data was assessed using R programming language (pROC, qreg, and Stan and loo packages). Expression of *SOCS1-3* and *SOCS5* genes was calculated using Ct and efficiency. Values were log-transformed. The normality of data was assessed using Shapiro–Wilk test. Since data was non-parametric, Mann–Whitney U test was used for comparison of mean values. Spearman correlation coefficient was measured to estimate correlation between expressions of *SOCS1-3* and *SOCS5* genes. Diagnostic power of these genes was assessed through depicting receiver operating characteristic curves using pROC-package. The level of statistical significance was set at $P < 0.05$.

Results

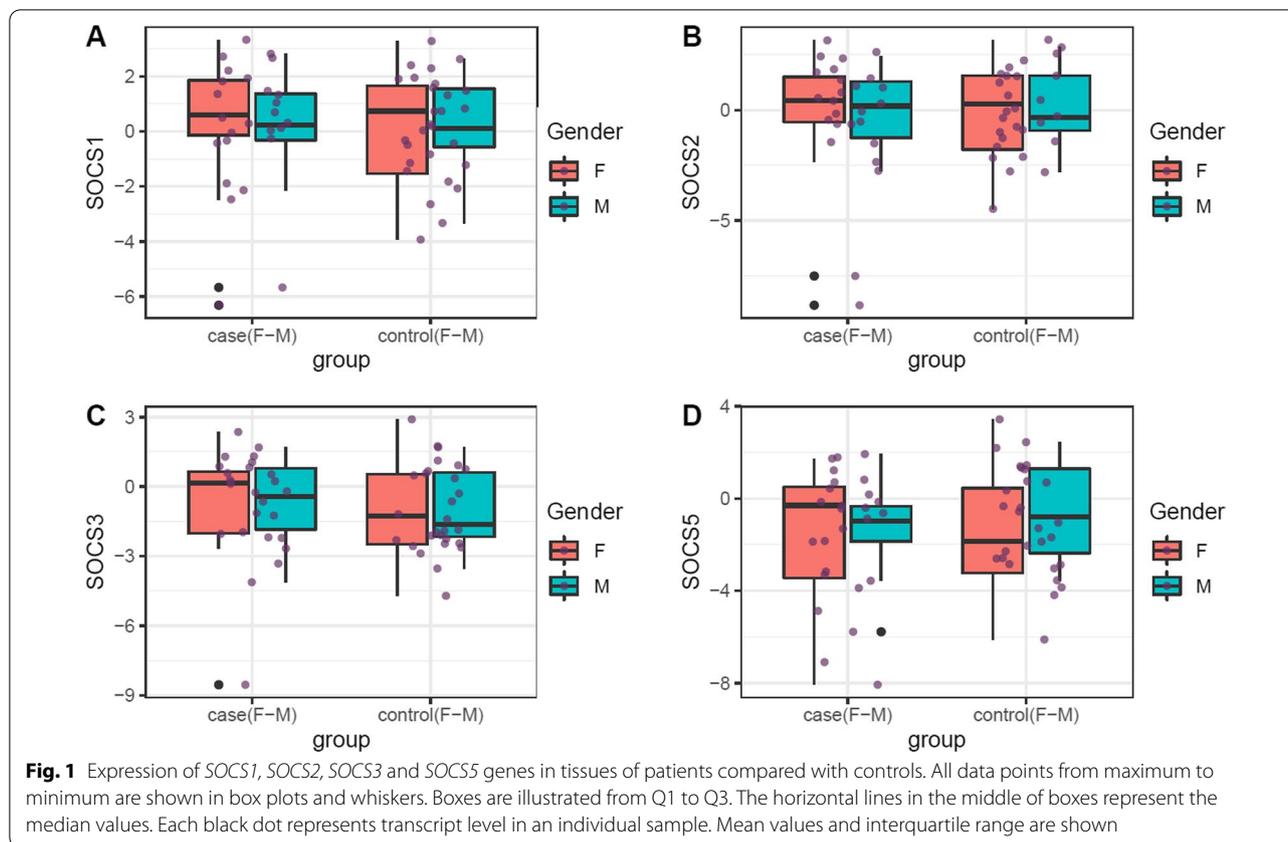
General data

A total of 26 patients with periodontitis (Female/male ratio: 16/10) and 28 controls (female/male ratio: 12/16) were enrolled. Cases and controls were matched in terms of their age (Mean age \pm SD: 37.6 \pm 2.5 versus 37.5 \pm 1.7).

Experiments

Expressions of *SOCS1*, *SOCS2*, *SOCS3* and *SOCS5* genes in tissue and blood of study subgroups are shown in Figs. 1 and 2, respectively.

Expressions of *SOCS1*, *SOCS2*, *SOCS3* and *SOCS5* genes were similar between gingival tissues of patients and controls. However, expression of *SOCS1* was lower in blood samples of patients in comparison with unaffected individuals (Ratio of mean expression (RME) = 0.47, P value = 0.04). The same pattern



was observed among female subjects (RME=0.38, P value=0.04). *SOCS2* was under-expressed in blood samples of female patients compared with female controls (RME=0.22, P value=0.04). *SOCS3* was also under-expressed in the circulation of total patients compared with total controls (RME=0.29, P value=0.02) and in female patients compared with female controls (RME=0.19, P value=0.04). Blood levels of *SOCS5* were not changed between subgroups (Table 1).

While expressions of *SOCS* genes were strictly correlated in each set of samples, no correlation was found between their tissue and blood levels (Fig. 3).

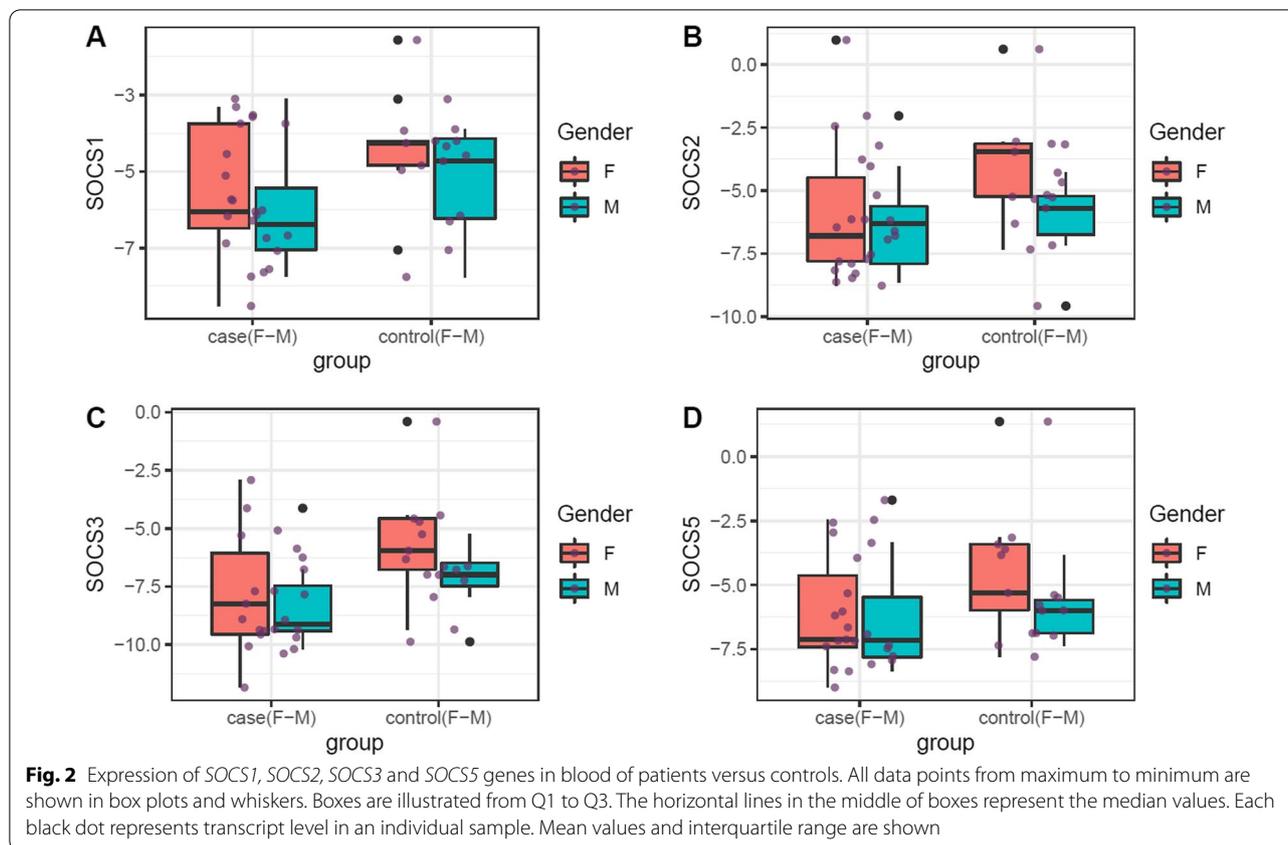
Finally, we measured the diagnostic power of *SOCS* genes in blood and tissue samples using the Bayesian Generalized Linear Model (Fig. 4).

SOCS2 had the best performance in separation of affected tissues from unaffected tissue samples (AUC=0.66, sensitivity=0.39, specificity=0.83). *SOCS3* was superior to other transcripts in differentiation of blood samples of cases from normal blood samples (AUC=0.69, sensitivity=0.81, specificity=0.68). Combination of transcript levels of *SOCS1*, *SOCS2*, *SOCS3* and *SOCS5* genes enhanced the AUC values to 0.64 and 0.67 in tissue and blood samples, respectively (Table 2).

Discussion

Periodontitis is a disorder with high burden on personal health. Metagenomics and periodontal flap design have been found to be risk factors of developing periodontitis [1, 2]. In an attempt to find some aspects of immune dysregulation in periodontitis, we assessed expression levels of *SOCS* genes in the blood and gingival tissues of periodontitis patients compared with matched controls. We found similar levels of *SOCS* genes between gingival tissues of patients and controls. Yet, we found under-expression of *SOCS1* in blood samples of patients compared with controls. *SOCS2* and *SOCS3* were down-regulated in blood samples of female patients compared with female controls. *SOCS3* was also under-expressed in the circulation of total cases versus total controls. Expression of *SOCS5* was not different between blood samples two study groups.

SOCS1 has been firstly recognized as a negative modulator of the JAK/STAT-mediated signaling pathway induced by IL-4, IL-6, and IFN- γ cytokines [14–16]. Several cytokines have been dysregulated in the course of periodontitis contributing in the pathoetiology of this condition. Most importantly, release of IL-6 and TNF family cytokines by periodontal and immune cells following stimulation by pathogens induces and attracts



specific immune cells causing tissue injury [17]. Release of IL-4 by naive CD4+ T cells and B cells has a role in activation of STAT6 and GATA3 and stimulation of differentiation of Th2 cells and B cells [18, 19]. A previous meta-analysis has indicated consistent down-regulation of IL-4 in patients with chronic periodontitis and elevation of its levels following treatment of this condition [20]. On the other hand, gingival crevicular fluid levels of IL-6 and IFN- γ have been found to be remarkably higher in these patients compared with healthy controls [20]. Another study has detected parallel up-regulation of *SOCS1* and IFN- γ in the experimental model of periodontitis [8]. These findings indicate the presence of a complicated network between cytokines, their regulatory factors and their mediators in the context of periodontitis. Down-regulation of *SOCS1* in the blood samples of patients with periodontitis might lead to up-regulation of several immune-related pathways and molecules which are regulated by this protein. The consequent up-regulation of activity of these pathways might affect the pathogenesis of periodontitis in the gingival tissues.

SOCS2 was under-expressed in blood samples of female patients compared with female control group. This member of SOCS protein family has a fundamental role in the regulation of balance between immune responses and

oxidative stress [21]. Thus, dysregulation of this protein might affect the regenerative processes in the course of periodontitis.

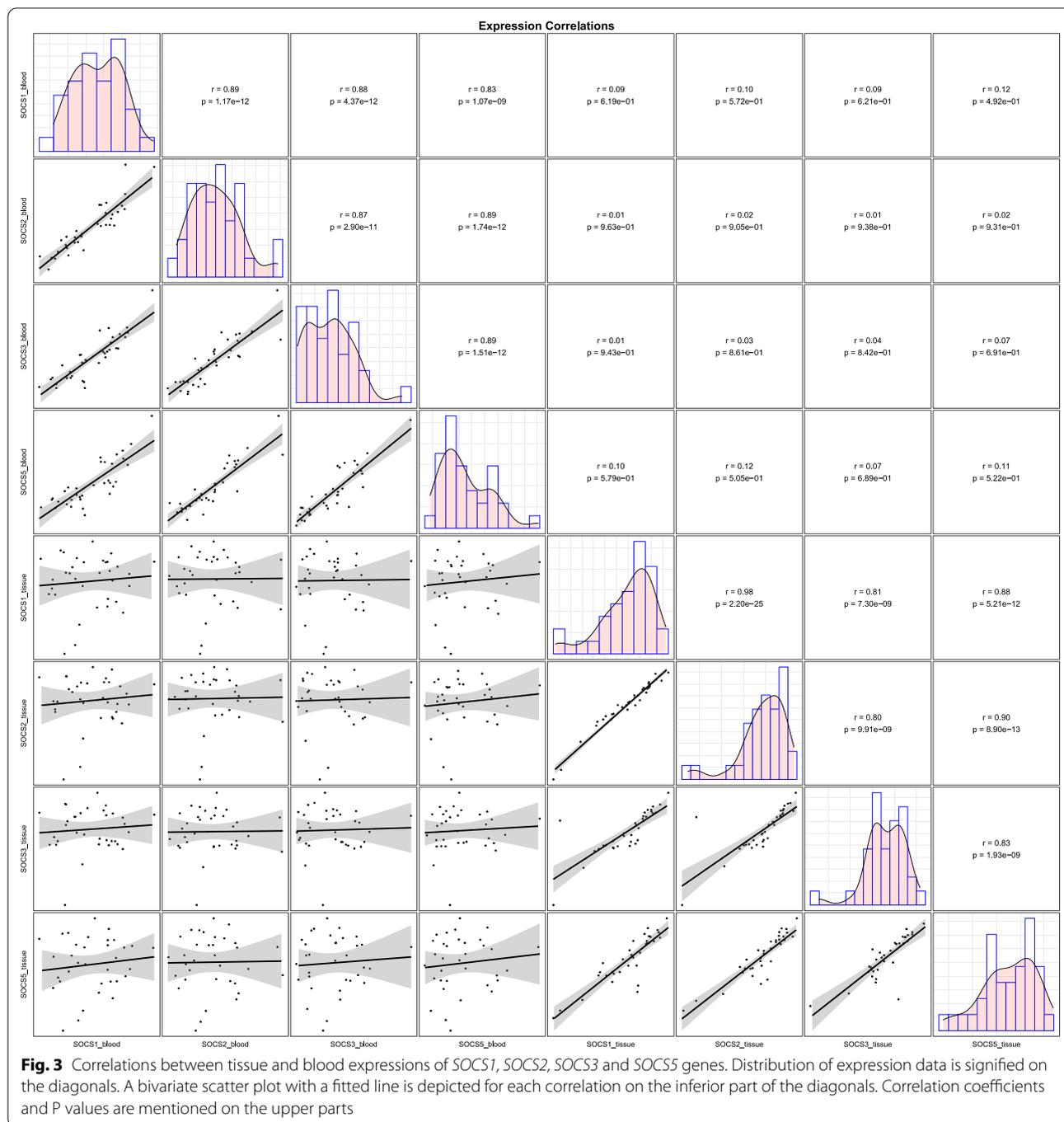
SOCS3 was also under-expressed in the circulation of total patients versus total control group and in female patients compared with female control group. *SOCS3* is regarded as an important negative regulator of alveolar bone loss in periodontitis [9]. Thus, down-regulation of *SOCS3* might lead to increased alveolar bone loss in these patients.

The observed sex-biased alterations in the expressions of *SOCS* genes in the current study might be due to small number of male subjects in the current study. Alternatively, *SOCS* genes might exert a sex-based role in the pathogenesis of periodontitis.

The results of the current study are in line with other studies reporting abnormal expressions of immune-related genes in the blood of patients with periodontitis. For instance, two NF- κ B-related genes, namely *DILC* and *FBXL19-AS* have been shown to be down-regulated both in blood samples of these patients compared with control samples [22]. A number of other long non-coding RNAs, namely *Linc0116*, *Linc00667*, *CDK6-AS1*, *FENDRR* and *DIRC3* have been found to be down-regulated in the blood samples of patients with periodontitis [23].

Table 1 Assessment of expression of SOCS1, SOCS2, SOCS3 and SOCS5 genes in tissues and blood specimens of cases versus controls

Number of Samples	SOCS1					SOCS2					SOCS3					SOCS5					
	SE	RME	P value	95% CI		SE	RME	P value	95% CI		SE	RME	P value	95% CI		SE	RME	P value	95% CI		
<i>Tissues</i>																					
Total	26/28	0.58	1.05	0.91	-1.10	1.24	0.66	0.88	0.78	-1.50	1.14	0.57	1.20	0.65	-0.88	1.40	0.69	0.71	0.47	-1.89	0.88
F	16/12	0.93	1.01	0.99	-1.89	1.91	1.05	0.83	0.80	-2.42	1.89	0.89	1.18	0.79	-1.60	2.07	1.11	0.85	0.84	-2.51	2.05
M	10/16	0.68	1.16	0.76	-1.21	1.63	0.70	1.04	0.93	-1.39	1.52	0.74	1.21	0.71	-1.28	1.83	0.87	0.68	0.53	-2.39	1.29
<i>Blood</i>																					
Total	23/17	0.50	0.47	0.04	-2.11	-0.08	0.76	0.42	0.11	-2.80	0.30	0.72	0.29	0.02	-3.25	-0.32	0.70	0.50	0.16	-2.43	0.41
F	15/10	0.63	0.38	0.04	-2.71	-0.09	0.97	0.22	0.04	-4.19	-0.17	1.04	0.19	0.04	-4.56	-0.17	0.98	0.33	0.12	-3.67	0.47
M	8/7	0.79	0.60	0.37	-2.43	0.96	1.03	1.02	0.97	-2.18	2.25	0.89	0.47	0.24	-3.04	0.84	0.96	0.88	0.85	-2.31	1.94



Finally, we appraised diagnostic power of *SOCS* transcripts in the context of periodontitis. *SOCS2* had the best performance in separation of affected tissues from unaffected ones. *SOCS3* was superior to other transcripts in differentiation of blood samples of patients from normal blood samples. Integration of transcript levels of *SOCS1*, *SOCS2*, *SOCS3* and *SOCS5* genes improved the AUC values in both tissue and blood samples.

Taken together, *SOCS1*, *SOCS2* and *SOCS3* genes have been found to be dysregulated in the circulation of patients with periodontitis. These transcripts might be regarded as peripheral markers for periodontitis. Yet, tissue levels of these transcripts were similar between two study subgroups, indicating that these transcripts possibly regulate systemic but not local immune responses in this condition. Our study has a limitation regarding

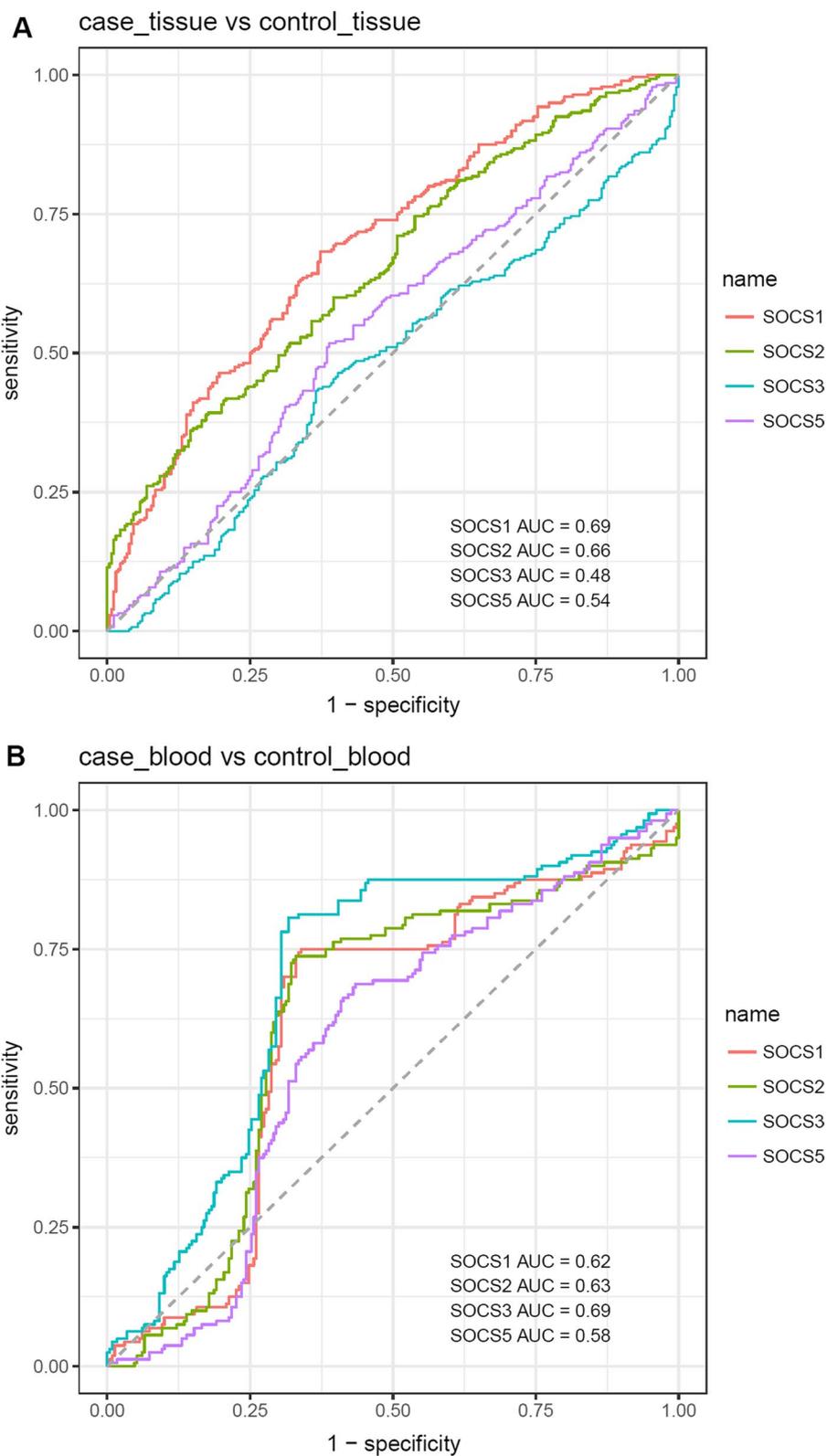


Fig. 4 Bayesian generalized linear model-based ROC curves

Table 2 ROC curves-related statistics in tissue and blood samples

Samples	SOCS1			SOCS2			SOCS3			SOCS5			All		
	AUC	Sensitivity	Specificity	AUC	Sensitivity	Specificity									
Tissue	0.69	0.68	0.63	0.66	0.39	0.83	0.48	0.43	0.63	0.54	0.52	0.61	0.64	0.71	0.52
Blood	0.62	0.75	0.66	0.63	0.74	0.67	0.69	0.81	0.68	0.58	0.69	0.57	0.67	0.78	0.65

the sample size. Thus, we propose conduction of further studies with larger sample sizes for verification of the results of the current study. Particularly, the obtained AUC values should be verified in independent studies. Future *in vitro* studies are also needed to evaluate the function of *SOCS* genes in the pathophysiology of periodontitis.

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Author contributions

SGF wrote the manuscript and revised it. MT and AS supervised and designed the study. SS, LG and BMH collected the data and performed the experiment. NN analyzed the data. All authors read and approved the submitted version.

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Availability of data and materials

All data generated or analysed during this study are included in this published article.

Declarations

Ethics approval and consent to participant

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent forms were obtained from all study participants. The study protocol was approved by the ethical committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.DRC.REC.1400.012). All methods were performed in accordance with the relevant guidelines and regulations.

Consent of publication

Not applicable.

Competing interests

The authors declare they have no competing interests.

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