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Sex-based Dysregulation of Inflammation-related Genes in Periodontitis

Soudeh Ghafouri-Fard[1], Leila Gholami[2], Elham Badrlou[1,3], Saba Sadeghpour[1,4], Naghme Nazer[5], Mahdi Shadnoush[6], Sheyda Khalilian[1], Arezou Sayad[7,1]*

1. Department of Medical Genetics, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2. Department of Periodontics, Dental Research Center, Hamadan University of Medical Sciences, Hamadan, Iran.
3. Pediatric Cell Therapy Research Center, Tehran University of Medical Sciences, Tehran, Iran.
4. Hematopoietic Stem Cell Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
5. Department of Electrical Engineering, Sharif University of Technology, Tehran, Iran.
6. Department of Clinical Nutrition, Faculty of Nutrition and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
7. Dental Research Center, Research Institute for Dental Sciences, Dental School, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

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ABSTRACT

Periodontitis is a chronic inflammatory condition affecting a large population all over the world. This condition is linked with abnormal expression of numerous genes. We measured levels of *CYFIP1*, *KDR*, *RABGGTA*, *RABGGTB* and *FOXD2* in gingival tissue and circulation of people with periodontitis and healthy controls. *KDR* was more expressed in tissue samples of female patients compared with female controls (Ratio of mean expression (RME) =4.16, P=0.02). However, this gene was less expressed in the blood of female patients compared with female control subjects (RME=0.12, P=0.04). *RABGGTB* was less expressed in the blood of male patients compared with male controls (RME=0.20, P=0.02). Finally, *FOXD2* was less expressed in total blood samples compared with total controls (RME=0.3, P<0.001) and in blood samples of female patients compared with female control subjects (RME=0.02, P<0.001). *RABGGTA* had the best area under curve (AUC) value in differentiation of patients' tissues from normal tissues (AUC=0.60, sensitivity=0.37, specificity=0.92). In distinction of abnormal blood samples from controls, *FOXD2* had the best performance (AUC=0.85, sensitivity=0.66, specificity=0.91). In brief, we demonstrated a sex-dependent dysregulation of *KDR*, *RABGGTB* and *FOXD2* genes in circulation or tissue of patients with periodontitis.

Keywords: Cyfipi, KDR, RABGGTA, RABGGTB, FoxD2, periodontitis

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*Corresponding: Arezou Sayad
Address: Dental Research Center, Research Institute for Dental Sciences, Dental School, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
E-mail: ar.sayad@yahoo.com

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Introduction

Periodontitis is an inflammatory condition initiated by commensal microorganisms of the oral cavity but persisted due to host responses (1). Pathogenic composition of the biofilm has an essential role in the establishment of periodontal disorders (2). This condition has been found to be regulated by host inflammatory responses. It is worth mentioning that gender has a role in determining oral health (3). Genetic factors contribute to the risk of periodontitis. A previous study in monozygotic and dizygotic twins has proposed that genetic factors can explain approximately half of the variance in periodontitis risk (4). Consistent with this estimation, several polymorphisms within immune response-related genes have been found that influence susceptibility to periodontitis in different populations (5, 6). The underlying mechanism of involvement of several of these genes in the pathoetiology of periodontitis has been evaluated (5, 6). Yet, a number of other genes with putative roles in the regulation of immune response have been less studied.

CYFIP1 is a protein that controls apical-basal polarity during the neurodevelopmental processes (7). This protein has been shown to be directly controlled by NOTCH pathway (8), a signaling pathway whose down-regulation participates in the extent of bone destruction in aggressive periodontitis (9). Kinase insert domain receptor (KDR) is a receptor for VEGF (10), a multifunctional angiogenic cytokine that participates in inflammatory responses, tissue regeneration as well as pathogenesis of periodontitis (11). RABGGTA and RABGGTB genes encode subunits of the prenyltransferase RabGGTase. Protein prenylation has been displayed to confine innate immune responses through suppressing Rac1 effector interactions (12). Finally, FOXD2 is a gene attributed to the forkhead transcription factors with no specific recognized function.

To assess unexplored research areas in this field, we measured levels of CYFIP1, KDR, RABGGTA, RABGGTBand FOXD2 in gingival tissue and circulation of patients with periodontitis and normal controls.

Materials and methods

This study is a case-control study. Sex and age of cases were recorded in datasheets. Since there was no data about the expression levels of mentioned genes in periodontitis, this study was conducted as a pilot study. Blood samples were collected from cases and controls in EDTA tubes and transferred to the laboratory in liquid nitrogen. Blood and tissue samples were then stored in -70 C until the RNA extraction step.

Sampling

Gingival specimens were acquired from patients with chronic periodontitis, according to the standards explained in a previous investigation (13). In brief, the following criteria were regarded as inclusion criteria: chronic periodontitis (stage II-IV), probing depth of 5 mm or greater, and a minimum of 3 mm of attachment loss. Cigarette smoking, alcohol drinking, history of immune-related disorders, cancers, diabetes mellitus and consumption of antibiotics or anti-inflammatory agents were described as criteria for excluding patients. All samples were excised during surgical processes performed in the periodontal clinics. Control samples were acquired from healthy persons in the course of dental crown lengthening during 2022. Controls had no sign of periodontitis or other inflammatory conditions. The study protocol was approved by the Ethics

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Committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.DRC.REC.1400.019). All cases and controls signed the informed consent form.

Expression assay

PicoPure™ RNA Isolation Kit (Thermo Fisher Scientific, London, UK) was used for extraction of RNA from gingival and blood samples. First strand cDNA was produced from RNA templates by using the cDNA production kit (Smobio Company, Taiwan).CYFIP1, KDR, RABGGTA, RABGGTB and FOXD2 were quantified in gingival and blood samples using the GeneDireX kit (Taiwan). B2M was used as the reference gene. Reactions were performed in the LightCycler® 96 instrument. Primer sequences are displayed in Table 1.

Table 1. Primer Sequences.

Gene name	Sequence
B2M (Reference gene)	F: AGATGAGTATGCCTGCCGTG R: GCGGCATCTTCAAACCTCCA
CYFIP1	F: CACTGGGCTGGCTGTATGATC R: GACTTTAAGTAGATGGTAGCAGAAATCC
KDR	F: CTACTGATTTTTGCCCTTGTTC R: TAGTCATTGTTCCCAGCATTTC
RABGGTA	F: GGGCAACGTATCTGGATGACC R: AGAGCACTGTCAGATCCTTGTG
RABGGTB	F: CGGAGAAGTTACCAGATGTATGC R: TACGCAGTTTCTCTCTATCAATCC
FOXD2	F: CTGCGCCAAAGCCTTCTA R: TGGCCCATGATGTGGTCTAT

Statistical analysis

Statistical parameters were studied in the R environment. Expressions of CYFIP1, KDR, RABGGTA, RABGGTB and FOXD2 mRNAs were measured using cycle threshold and PCR efficiencies for each primer set following log-transforming of the raw data. Mean values of expression levels of CYFIP1, KDR, RABGGTA, RABGGTB and FOXD genes were compared between cases and controls using t-test. Correlations between expressions of CYFIP1, KDR, RABGGTA, RABGGTB and FOXD genes were evaluated using Spearman correlation coefficient. Diagnostic power of CYFIP1, KDR, RABGGTA, RABGGTB and FOXD transcripts was assessed through depicting receiver operating characteristic curves (ROC) and estimation of the area under curves (AUC).

Results

The current project was performed on specimens acquired from 16 female and 10 male patients with periodontitis and 12 female and 16 male control subjects. Mean age (±standard deviation) was 37.6±2.5 years and 37.5±1.7 years in cases and controls, respectively.

Experiments

Relative expressions of CYFIP1, KDR, RABGGTA, RABGGTB and FOXD2 genes in tissues and blood samples of cases and control subjects are presented in Figures 1 and 2, respectively.

KDR was more expressed in tissue samples of female patients compared with female controls (Ratio of mean expression (RME) =4.16, P=0.02). However, this gene was less expressed in blood samples of

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female patients compared with controls (RME=0.12, P=0.04). RABGGTB was less expressed in the blood of male patients compared with male controls (RME=0.20, P=0.02). Finally, FOXD2 was less expressed in total

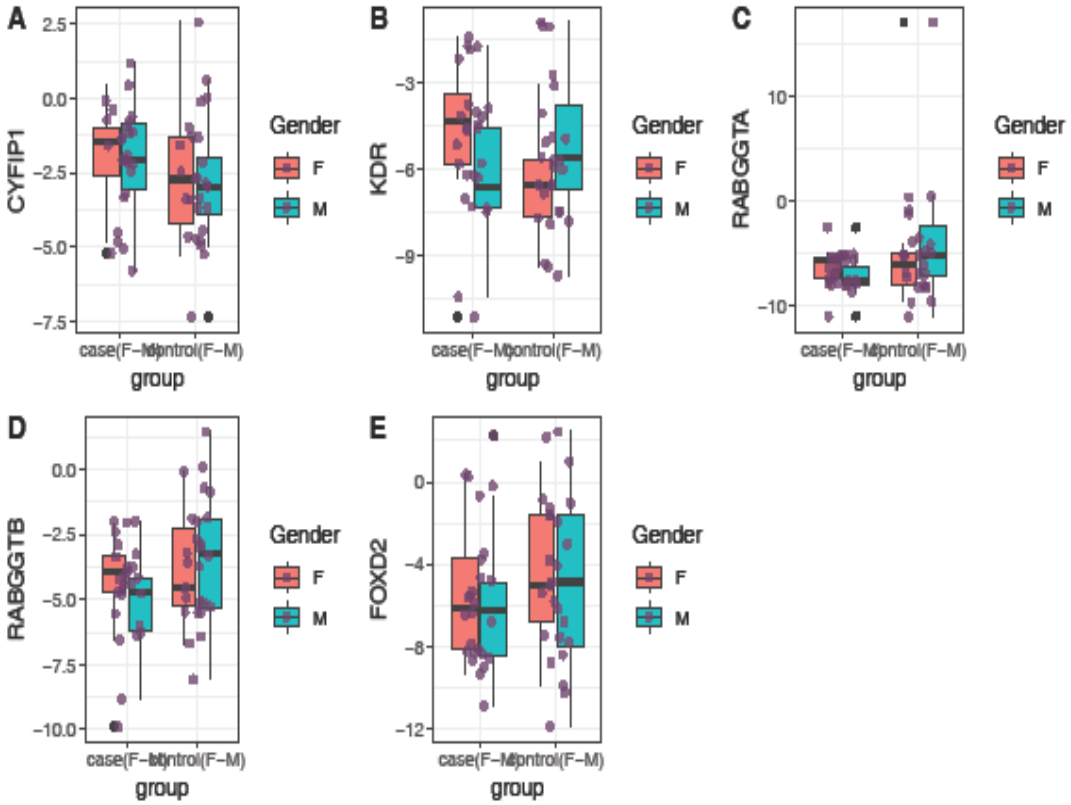
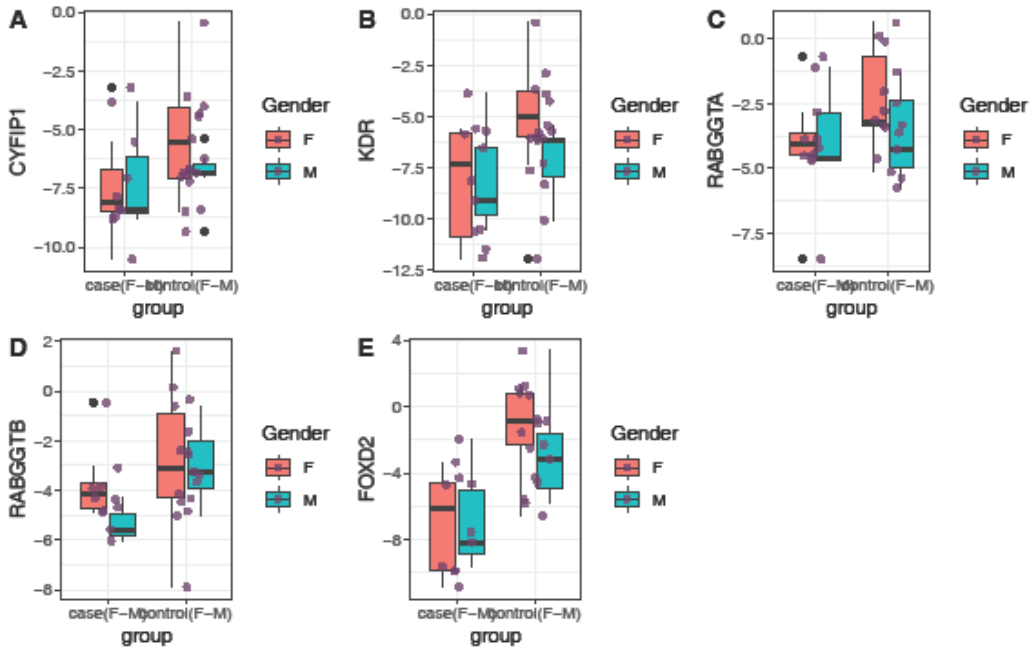


Fig.1. Expression of *CYFIP1*, *KDR*, *RABGGTA*, *RABGGTB* and *FOXD2* in affected tissues versus control tissues.



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Fig.2. Expression of *CYFIP1, KDR, RABGGTA, RABGGTB* and *FOXD2* in blood samples of patients with periodontitis versus controls.

blood samples compared with total controls (RME=0.3, P<0.001) and in blood samples of female patients compared with female controls (RME=0.02, P<0.001). Expressions of other genes were similar between tissue/blood of two groups (Table 2).

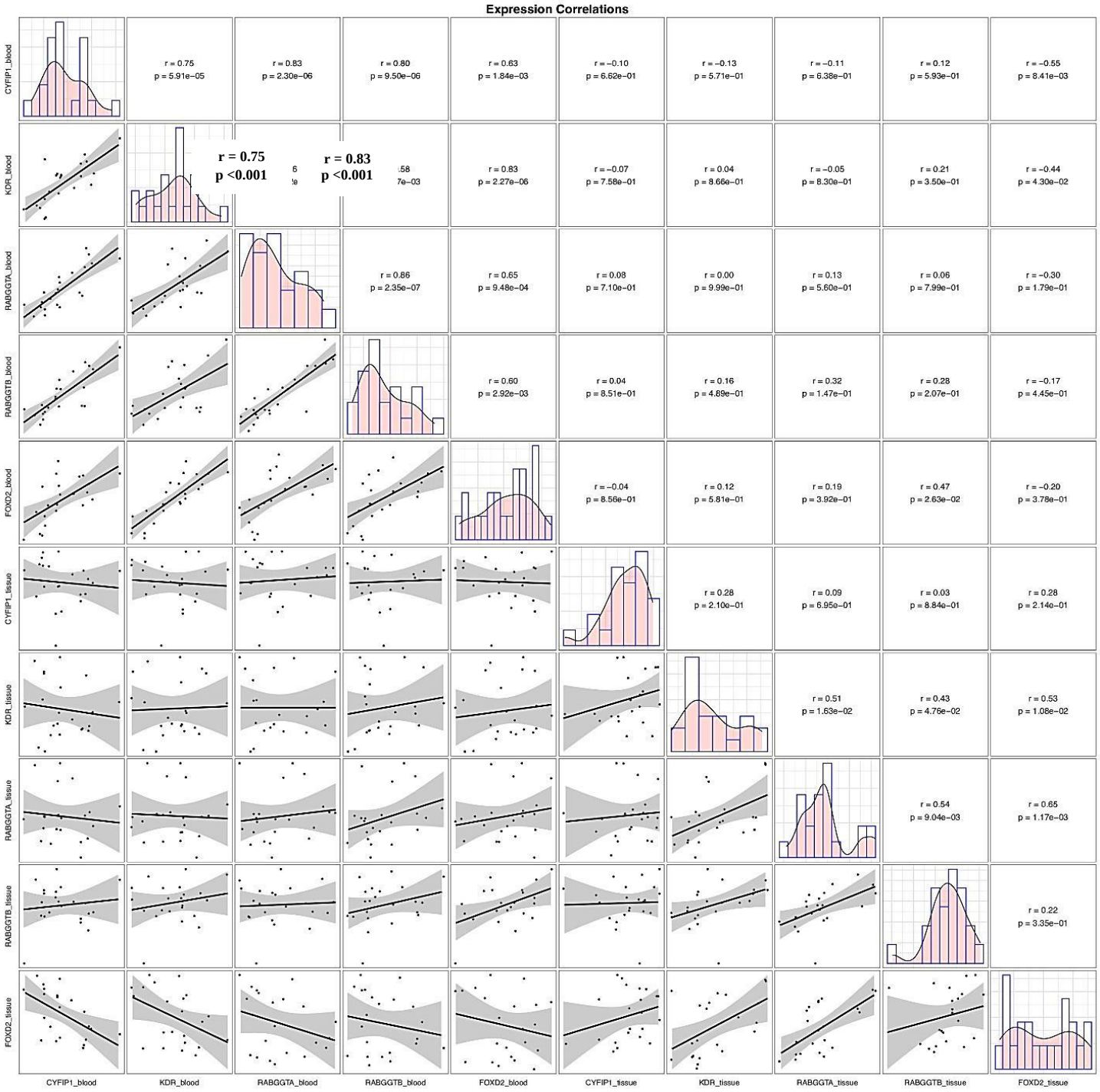
Table 2. Statistical values of expression analysis of *CYFIP1, KDR, RABGGTA, ABGGTB* and *FOXD2* genes in tissues and blood specimens acquired from patients compared with controls (RME: ratio of mean expression).

	CYFIPI					
	sampies	**SE**	**RME**	**P.VAIUE**	**95%c**	
Tissues	TOTAI	0.55	1.63	0.20	-0.40	1.81
	F	0.83	1.38	0.58	-1.28	2.21
	M	0.85	1.78	0.34	-0.95	2.61
Blood	TOTAI	0.86	0.41	0.15	-3.07	0.50
	F	1.12	0.25	0.09	-4.40	0.36
	M	1.66	0.94	0.96	-6.32	6.14
	KDR					
	sampies	SE	RME	P.VAIUE	95%c	
Tissues	TOTAI	0.68	1.55	0.36	-0.73	2.01
	F	0.84	4.16	0.02	0.33	3.79
	M	1.03	0.53	0.38	-3.06	1.22
Blood	TOTAI	1.06	0.23	0.06	-4.33	0.06
	F	1.36	0.12	0.04	-5.92	-0.14
	M	2.11	0.60	0.76	-8.51	7.04
	RABGGTA					
	sampies	SE	RME	P.VAIUE	95%c	
Tissues	TOTAI	1.11	0.26	0.09	-4.18	0.34
	F	2.25	0.27	0.42	-689	3.09
	M	1.16	0.22	0.07	-459	0.22
Blood	TOTAI	0.77	0.50	0.21	-2.61	0.61
	F	0.98	0.30	0.10	-3.83	0.36
	M	1.34	1.19	0.86	-3.81	4.32
	RABGGTA					
	sampies	SE	RME	P.VAIUE	95%c	
Tissues	TOTAI	0.59	0.47	0.07	-2.27	0.11
	F	0.79	0.66	0.44	-2.28	1.03
	M	0.92	0.33	0.10	-3.53	0.31
Blood	TOTAI	0.71	0.41	0.06	-2.75	0.18
	F	1.02	0.53	0.39	-3.09	1.28
	M	0.79	0.20	0.02	-4.22	-0.47
	FOXD2					
	sampies	SE	RME	P.VAIUE	95%c	-
Tissues	TOTAI	1.02	0.52	0.36	-299	-
	F	1.30	0.51	0.46	-3.68	-
	M	1.67	0.49	0.55	-450	-
Blood	TOTAI	1.17	0.03	0.00	-7.38	-
	F	1.35	0.02	0.00	-8.48	-
	M	2.64	0.07	0.23	-12.17	

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Expressions of CYFIP1, KDR, RABGGTA, RABGGTB and FOXD2 genes were strongly correlated with each other in each set of clinical samples (gingival tissues or venous blood), yet there was no significant correlation between their expressions in gingival tissues and blood samples (Figure 3).



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Fig.3. Correlation between gingival/ blood amounts of *CYFIP1, KDR, RABGGTA, RABGGTB* and *FOXD2* genes. Distribution of parameters is shown on the diagonals .Bivariate scatter graphs with a fitted line are shown below the diagonals. Correlation coefficients and P values are shown in the upper sections.

We quantified the diagnostic power of CYFIP1, KDR, RABGGTA, RABGGTB and FOXD2 genes in blood and gingival specimens using the Bayesian Generalized Linear Model (Figure 4).

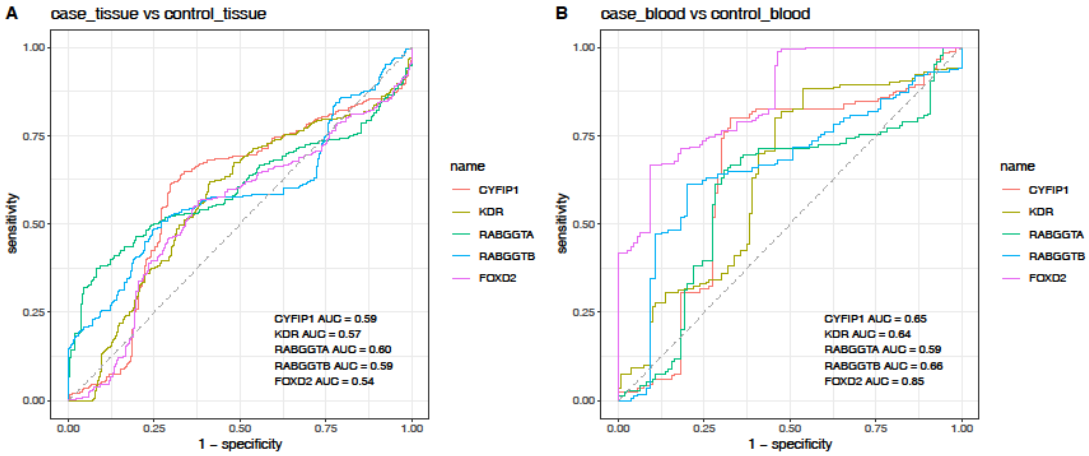


Fig. 4. ROC curves depicted using the Bayesian Generalized Linear Model.

RABGGTA had the best AUC value in separation of abnormal tissues from normal tissues (AUC=0.60, sensitivity =0.37, specificity=0.92). Combination of expression levels of CYFIP1, KDR, RABGGTA, RABGGTB and FOXD2 genes improved AUC and sensitivity amounts to 0.77 and 0.84, respectively.

In distinction of blood samples of patients from control individuals, FOXD2 had the best performance (AUC=0.85, sensitivity=0.66, specificity=0.91). Combination of expression levels of CYFIP1, KDR, RABGGTA, RABGGTB and FOXD2 genes resulted in AUC, sensitivity and specificity amounts of 0.78, 0.99 and 0.55, respectively (Table 3).

Table 3. Statistical parameters of ROC curves in tissue and blood samples.

	CYFIP1			*KDR*			*RABGGTA*			*RABGGTB*			*FOXD2*			All		
Sample type	**AUC**	**Sensitivity**	**Specificity**	**AUC**	**Sensitivity**	**Specificity**	**AUC**	**Sensitivity**	**Specificity**	**AUC**	**Sensitivity**	**Specificity**	**AUC**	**Sensitivity**	**Specificity**	**AUC**	**Sensitivity**	**Specificity**
Tissue	0.59	0.61	0.70	0.57	0.62	0.59	0.60	0.37	0.92	0.59	0.51	0.73	0.54	0.57	0.62	0.77	0.84	0.58
Blood	0.65	0.80	0.67	0.64	0.88	0.46	0.59	0.65	0.69	0.66	0.61	0.80	0.85	0.66	0.91	0.78	0.99	0.55

Discussion

Several genes have been previously found to be dysregulated in periodontitis (14, 15). However, the role of several other genes in this process has been less studied. We quantified levels of CYFIP1, KDR, RABGGTA, RABGGTB and FOXD2 in gingival tissue and circulation of patients with periodontitis and healthy controls.

KDR was over-expressed in tissue samples of female patients compared with female controls. However, expression of this gene was lower in the blood of female patients compared with female controls. KDR encodes a receptor for VEGF (10, 16), a growth factor and cytokine with presumed role in the

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pathoetiology of periodontitis. A previous study has reported higher volume of gingival crevicular fluid (GCF) and total concentrations of VEGF gathered from affected regions in patients with periodontitis compared with from clinically normal sites. VEGF level has been elevated in saliva of patients compared with healthy persons. Taken together, VEGF might be involved in the angiogenic processes in normal and affected periodontal tissues and the health status of periodontal tissue can affect the salivary levels of this factor (11). Another study has demonstrated an increasing trend in VEGF level in GCF from health to periodontitis. Moreover, treatment of periodontal disease can reduce its levels, indicating a role for VEGF in the progression of periodontal disorder (17). Furthermore, an in vitro study has indicated elevation of VEGF-A-related angiogenic differentiation in gingival fibroblasts and its association with induction of MAPK activity (18). Most importantly, a recent meta-analysis and evaluation of microarray data have shown the impact of high levels of VEGF in the development of periodontitis (19).

The observed discrepancy between tissue and blood levels of KDR might indicate specific tissue-based roles for this receptor in the context of periodontitis. Although the sex-specific role of KDR has not been assessed in previous studies, serum levels of VEGF have been found to be higher in females compared with leading to enhancement of cell growth and development among females (20). On the other hand, a more recent study has indicated higher levels of synovial VEGF in males affected with rheumatoid arthritis compared with female patients (21). Taken together, these studies indicate gender-based differences in VEGF functions.

RABGGTB was less expressed in blood samples of male patients compared with male controls. We have earlier described down-regulation of RABGGTB in female patients with multiple sclerosis compared with controls (22). These findings imply contribution of this protein in modulation of immune responses. Meanwhile, they show possible effects of sex-based factors on its expression in different conditions.

Finally, FOXD2 was under-expressed in total blood samples compared with total controls and in blood samples of female patients compared with female controls. The functional significance of this finding should be assessed in future studies.

RABGGTA had the highest AUC value in differentiating affected tissues from normal tissues. Combination of expression levels of CYFIP1, KDR, RABGGTA, RABGGTB and FOXD2 genes improved AUC and sensitivity values to 0.77 and 0.84, respectively. In differentiation of blood samples of patients from control specimens, FOXD2 had the highest values among the assessed genes. Combination of expression levels of CYFIP1, KDR, RABGGTA, RABGGTB and FOXD2 genes changed the AUC, sensitivity and specificity values of 0.78, 0.99 and 0.55, respectively. These findings indicate possible application of these transcripts in separation of affected samples from unaffected ones.

In brief, we confirmed a sex-dependent dysregulation of KDR, RABGGTB and FOXD2 genes in circulation or tissue of patients with periodontitis. Although the causal mechanism of such sex-based deregulation is not clear, previous studies have reported higher prevalence of periodontitis in males compared to females, signifying a possible sex-related bias in pathophysiology of periodontitis (23). Besides, a more recent study has shown higher possibility of periodontitis in males having lower bioavailable testosterone level (24). Future studies are needed to clarify the possible association between sex-based dysregulation of these genes and risk of periodontitis. We also state small sample size as a limitation of our study.

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