Received: 17 June 2021 | Revised: 18 July 2021 | Accepted: 20 July 2021

DOI: 10.1002/jcla.23926

RESEARCH ARTICLE

WILEY

Levels of SERPIN family proteins in peri-implant crevicular fluid in patients with peri-implantitis

Jianhui Jiang[1] | **Guanglin Gao**[2] | **Qingmei Wang**[3]

[1]Department of Implantology, School and Hospital of Stomatology, Fujian Medical University Fujian Stomatological Hospital, Fuzhou, China

[2]Rengde Outpatient Clinic, School and Hospital of Stomatology, Fujian Medical University Fujian Stomatological Hospital, Fuzhou, China

[3]Department of Stomatology, Fujian Medical University Union Hospital, Fuzhou, China

Correspondence
Qingmei Wang, Department of Stomatology, Fujian Medical University Union Hospital, NO.29, Xinquan Road, Fuzhou 350001, China.
Email: wangqm2410@hotmail.com.

Abstract

Purpose: Serine protease inhibitors (SERPINs) family has been discovered in many disorders with proteolysis mechanisms. Our study determined the SERPINBs protein expression via public-based GEO databases and further validated by peri-implant crevicular fluid (PICF) of peri-implantitis patients and healthy recruiters.

Methods: This study is a retrospective analysis. A total of 123 participants of Fujian Medical University Fujian Stomatological Hospital, consisting of 58 cases of peri-implantitis and 65 samples of healthy control were retrospectively analyzed by ELISA assays and explored the gene enrichment pathways and clinical significance of SERPINBs expression accompanied by two different cytokines (IL-6 and TNF-α). Moreover, the clinical significance of SERPINBs was evaluated in peri-implantitis patients with PICF samples by the receiver operating curve (ROC) using the area under the curve (AUC).

Results: KEGG database showed that Starch and sucrose metabolism, Retrograde endocannabinoid signaling, Prion diseases, Pentose phosphate pathways, and Olfactory pathways are up-regulated; GO database showed that synapse organization, synapse assembly, sequestering of triglyceride, sensory perception of smell, and regulation of synapse organization pathways are up-regulated. SERPINBs were overexpressed in peri-implant tissues and peri-implantitis patients with PICF. SERPINBs was positively correlated to IL-6 and TNF-α in peri-implantitis patients with PICF. The ROC-AUCs of SERPINBs achieved a significantly higher range from 0.895 to 0.939 in peri-implantitis patients with PICF. Therefore, certain SERPINBs expressions were not only perceived through PICF and peri-implant tissues but also showed potential significance in peri-implantitis.

Conclusion: SERPINBs play an influential role in the pathogenesis of peri-implantitis via binding with other inflammatory cytokines.

KEYWORDS
clade B, cytokines, peri-implantitis, PICF, SERPINs

Jianhui Jiang and Guanglin Gao authors contribute equally to this work.

1 | INTRODUCTION

In the current scenario with a high success rate, dental implants have become well established for patients suffering from tooth loss.[1] However, the occurrence of inflammation around implants has been reported more constantly and symptoms vary in severity and frequency.[2,3] Peri-implantitis is defined as an inflammatory reaction in the soft tissue near an implant that is reversible, or/and with the loss of supporting bone around an implant, which is irreversible.[4,5] 28% to 56% of patients have symptoms of peri-implant mucositis, meanwhile, 12% to 43% of patients were found bone loss and bleeding after 5–23 years of implants.[6] Accumulation of plaque, inflammation, and cytokines release subsequently, followed by alveolar bone decomposition, are cogitation as the main pathological processes for the development of peri-implantitis.[4,5,7]

Serine protease inhibitors (SERPINs), a superfamily of protease inhibitors, are responsible for lots of regulated processes such as coagulation, fibrinolysis, and inflammation.[8,9] SERPINs are important elements in regulating proteolytic pathways, controlling serine activity and preventing from excessive proteolysis.[10] More than 1500 members of SERPINs have been recorded till now and they exist in vertebrates, invertebrates, plants, bacteria.[11] Vertebrates SERPINs, with distinct exon-intron patterns, are classified into nine clades (A-I).[10,12]

At present, the extension of clade B SERPINs (SERPINBs) has been a vast catalog of functions in primarily mammals and animals due to the chain of duplication events. Subsequently in human 18q21.3 chromosome location, the newest duplicates of SERPINB3 and SERPINB4.[13] Likewise, these two isoforms genomic cloning disclosed that they were homologous or 91% similar at the amino acid level[14] and they share preserved tertiary structure. SERPINs utilize a unique formation rearrangement for inhibitory disruption. However, SERPINB3 and SERPINB4 display various properties. SERPINB3 is a papain-like cysteine proteinase inhibitor meanwhile SERPINB4 is a chymotrypsin-like SERPIN. Here, the SERPIN cluster commands the activity of various serine and cysteine protease.[15]

SERPINs family has been discovered in many disorders with proteolysis mechanisms. Nonetheless, the particular role of certain SERPINBs, SERPINB1, B3, B4, and B5 protein-coding genes, in peri-implantitis patients with peri-implant crevicular fluid (PICF), remains to be undetermined.

2 | MATERIALS AND METHODS

2.1 | Patients

This study is a retrospective analysis. A total of 123 cases of peri-implantitis patients and healthy control samples with PICF were retrospectively accumulated from Fujian Medical University Fujian Stomatological Hospital. The participants were enrolled from Feb 2020 to Mar. 2021. All subjects signed informed consent during their hospital stay, and the study was approved by the ethics committee of Fujian Medical University and was conducted following the Declaration of Helsinki guidelines.

2.2 | PICF extraction

The site was made moisture less by syringe (air) to removing the contamination of saliva, after insulating the implant with gauze. Then, PICF was possessed by putting 2 mm strips of standard paper into the pocket/sulcus for 30 s. The visually contaminated strips by blood were thrown away. PICF strips were inserted with 250 ml phosphate-buffered saline (PBS) and a cocktail of protease inhibitors (Beyotime, Shanghai, China) into microfuge tubes. Then, the samples were stored at −70°C for further analysis.

2.3 | ELISA assay

Peri-implant crevicular fluid samples were centrifuged for 15 min at 5000 g (4°C). Each PICF sample was analyzed by enzymatic immunosorbent assay (ELISA) assay to detect four SERPINBs and two cytokines' levels by following the recommendations and protocols of the manufacturer (Fine Test). At first, the detection antibody (100 ml) was put into wells, gently mixed, and overnight incubated at room temperature. Three times washed the plates. Samples and standards were inserted in all respective wells for the duplicate. The plates were cleaned again after the incubation time and inserted with conjugate (100 ml) at room temperature for 60 min. After plates were cleansed again by three times and substrate (100 ml) was inserted and incubated at room temperature for 15 min in the dark light. The stop solution of 50 ml was added and the reaction was stopped and measured of the color was done by microplate spectrophotometer (Microplate Reader, Bio-Rad). Results were quantified utilizing the standard curves from each assay.

2.4 | Bioinformatics

All data were acquired from the GEO database (http://www.ncbi.nih.gov/geo). The raw data were downloaded as MINiML files. The extracted data were normalized and processed by log2 transformation. The data were normalized using the preprocessCore package in R software (version 3.4.1) and Heat map, volcano map, box plots were drawn. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment pathways were analyzed using ClusterProfiler package in R software (version: 3.18.0).

2.5 | Statistical and datasets analysis

SPSS 20.0 version and GraphPad software were used for all the statistical analysis of the study and data were expressed as mean ± SD. The *t* test was performed for comparing the two

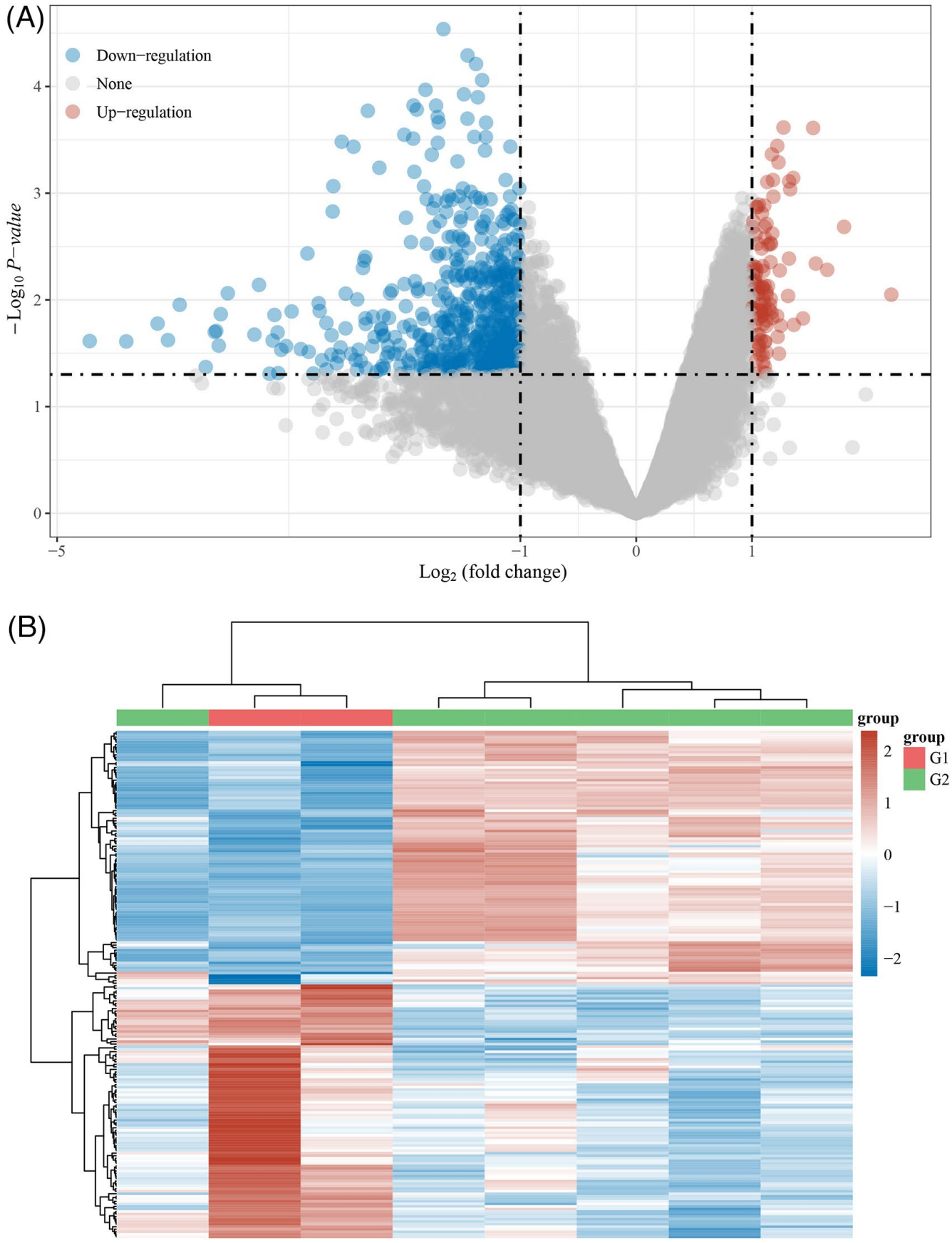

FIGURE 1 Bioinformatic analysis of GSE57631 comparing the peri-implantitis affected peri-implant tissue and healthy peri-implant tissue in humans. (A) Volcano plot is representing the dysregulated expression of genes. (B) Heat map is showing the up and down-regulated expressed genes in between two groups

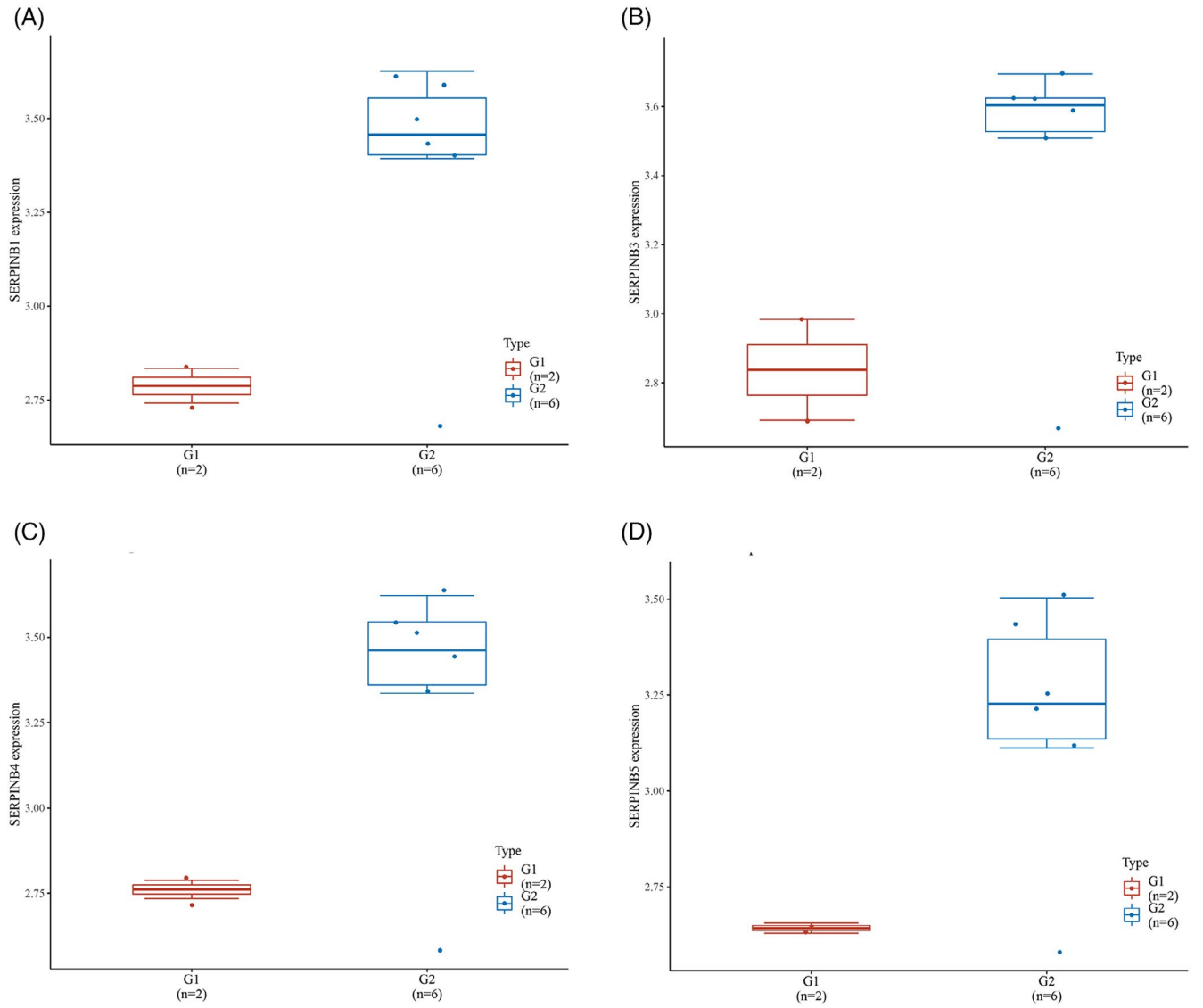

FIGURE 2 Comparison of the expression of four SERPINBs by GEO-based database. (A) SERPINB1, (B) SERPINB3, (C) SERPINB4, (D) SERPINB5 expressions were measured in two groups by box plots. G1: healthy peri-implant tissue; G2 peri-implantitis affected peri-implant tissue

groups. The pearson correlation was used for correlation analysis. The clinical significance of SERPINBs was evaluated in PICF by the receiver operating curve (ROC) using the area under the curve (AUC). $p < 0.05$ was considered to be representing statistically significant.

3 | RESULTS

3.1 | Discovery of four SERPINBs expressions in peri-implantitis

Bioinformatics tools were used to discover four SERPINBs (SERPINB1, B3, B4, and B5) protein-coding genes in between microarray datasets of peri-implantitis patients and healthy control of GEO database (GSE57631) via volcano plot, heat map, and box plot analyses (Figure 1A,B and Figure 2A-D). These heat map and volcano plot results show that these four SERPINBs protein-coding genes were differentially up-regulated significantly, which were further validated by the GSE57631 dataset of peri-implantitis affected tissues and healthy peri-implant tissues as control via box plots (Figure 2A-D). In the box plots, four SERPINBs expressions were elevated in peri-implantitis patients compared to controls significantly (Figure 2A-D, $p < 0.05$).

Moreover, the significant KEGG pathways of up-down-regulated genes were observed through the KEGG pathway database. Top-5 up-regulated pathways: Starch and sucrose metabolism, Retrograde endocannabinoid signaling, Prion diseases, Pentose phosphate pathways, and Olfactory; top-5 down-regulated pathways: Shigellosis, Salmonella infection, Protein processing in endoplasmic reticulum, Protein export, and Proteasome (Figure 3A, $p < 0.05$). Similarly, the GO database analysis represented the significant GO enriched

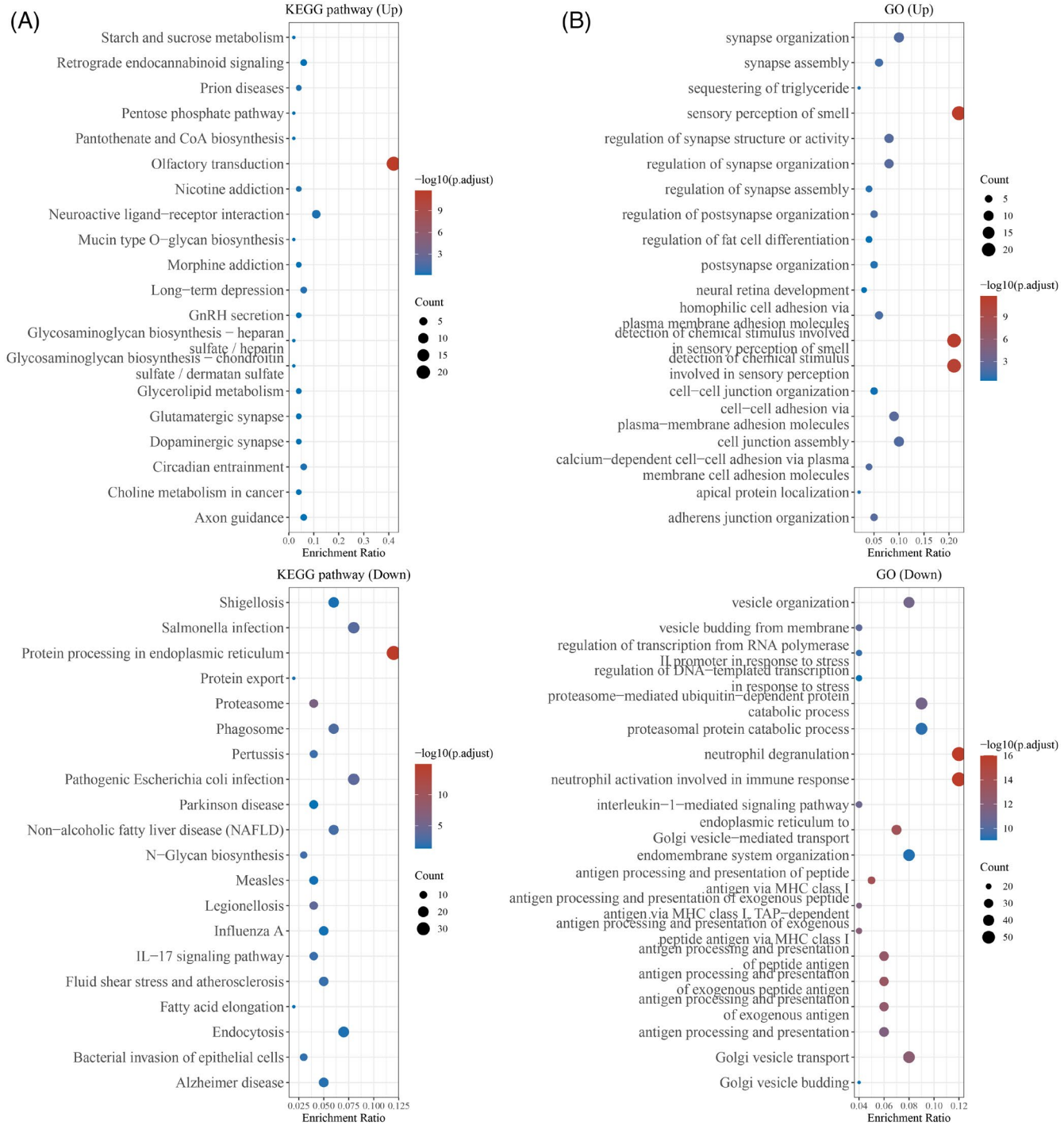

FIGURE 3 GO and KEGG pathways enrichment analysis. (A) The top significant KEGG pathways by enrichment analysis of targeted genes of peri-implantitis demonstrate similarly. (B) The topmost GO enrichment pathways of targeted genes of peri-implantitis show individually in up/down-regulated manners

pathways of up-down-regulated genes associated with peri-implantitis and healthy control groups. Top-5 up-regulated pathways: synapse organization, synapse assembly, sequestering of triglyceride, sensory perception of smell, and regulation of synapse organization; top-5 down-regulated pathways: vesicle organization, vesicle budding from membrane, proteasomal protein catabolic process, neutrophil degranulation, and neutrophil activation involved in immune response (Figure 3B, $p < 0.05$).

3.2 | Validation of four SERPINBs in PICF of peri-implantitis

To validate SERPINBs expression levels in PICF were shown by scatter plots in 58 peri-implantitis patients and 65 healthy controls. Herein, SERPINB1, SERPINB3, SERPINB4, and SERPINB5 expressions were notably up-regulated in PICF of peri-implantitis patients compared to the healthy control group (Figure 4A-D, $p < 0.001$).

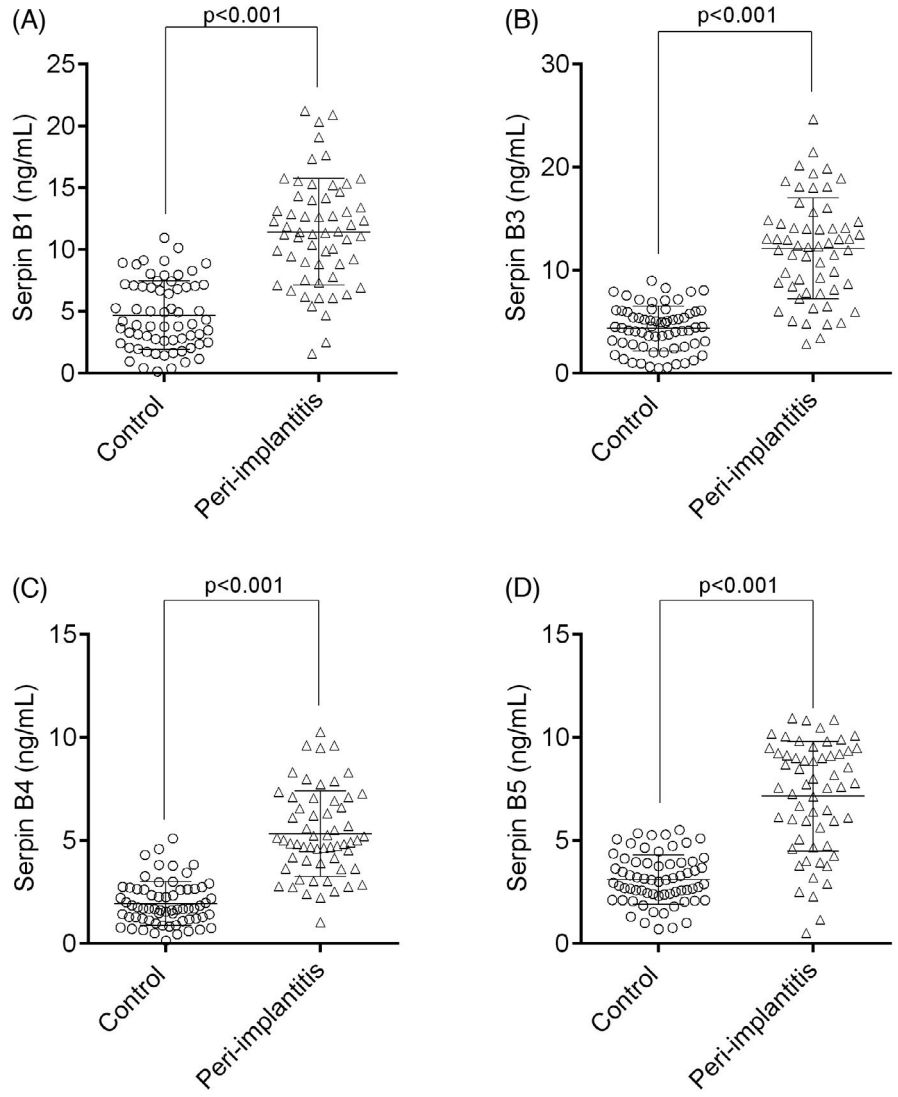

FIGURE 4 Comparison of the four SERPINBs in PICF of peri-implantitis and healthy controls. (A) SERPINB1, (B) SERPINB3, (C) SERPINB4, (D) SERPINB5 were analyzed by scatter plots

Therefore, the expressions of four SERPINBs were detectable in PICF of peri-implantitis patients.

3.3 | Significance of four SERPINBs in PICF of peri-implantitis

Besides, four SERPINBs significance were observed in peri-implantitis patients with PICF samples. Herein, the ROC-AUC of SERPINB1 and SERPINB3 achieved a significantly higher of 0.9021 (95% confidence interval (CI) = 0.8478~0.9565, $p < 0.05$) and 0.9268 (95%CI = 0.8798~0.9738, $p < 0.05$) in peri-implantitis with PICF samples, respectively (Figure 5A,B). Likewise, SERPINB4 in peri-implantitis patients achieved the highest AUC of 0.9398 (95%CI = 0.8991~0.9805, $p < 0.05$) among these four SERPINBs (Figure 5C). Subsequently, SERPINB5 in PICF of peri-implantitis patients yielded AUC of 0.8950 (95%CI = 0.8321~0.9578, $p < 0.05$), which was lowest compared to other SERPINBs (Figure 5D). Thus, these diagnostic values of four SERPINBs can accurately distinguish between peri-implantitis patients and healthy control groups with PICF, and further may provide potential significance in diagnosing peri-implantitis.

3.4 | IL-6 and TNF-α cytokines with SERPINBs in peri-implantitis

IL-6 and TNF-α, two different cytokines expression were demonstrated in PICF of peri-implantitis and healthy controls. Overall, these two different cytokines were highly expressed in peri-implantitis patients compared to healthy controls (Figure 6A,B, < 0.001). Meanwhile, SERPINBs correlation to IL-6 and TNF-α cytokines were observed via multiple scatter plots by Pearson's correlation analysis. Here, SERPINB1 was positively correlated to IL-6 ($r = 0.4796$, $p < 0.0001$) and TNF-α ($r = 0.4355$, $p < 0.0006$) (Figure 7A,B). While SERPINB3 was positively correlated to IL-6 ($r = 0.3906$, $p < 0.0024$) and TNF-α ($r = 0.3847$, $p < 0.0029$) (Figure 7C,D). Moreover, SERPINB4 was positively correlated to IL-6 ($r = 0.3429$, $p < 0.0084$) and TNF-α ($r = 0.3243$, $p < 0.0027$) (Figure 8A,B). Similarly, SERPINB5 was positively correlated to IL-6 ($r = 0.4063$, $p < 0.0016$)

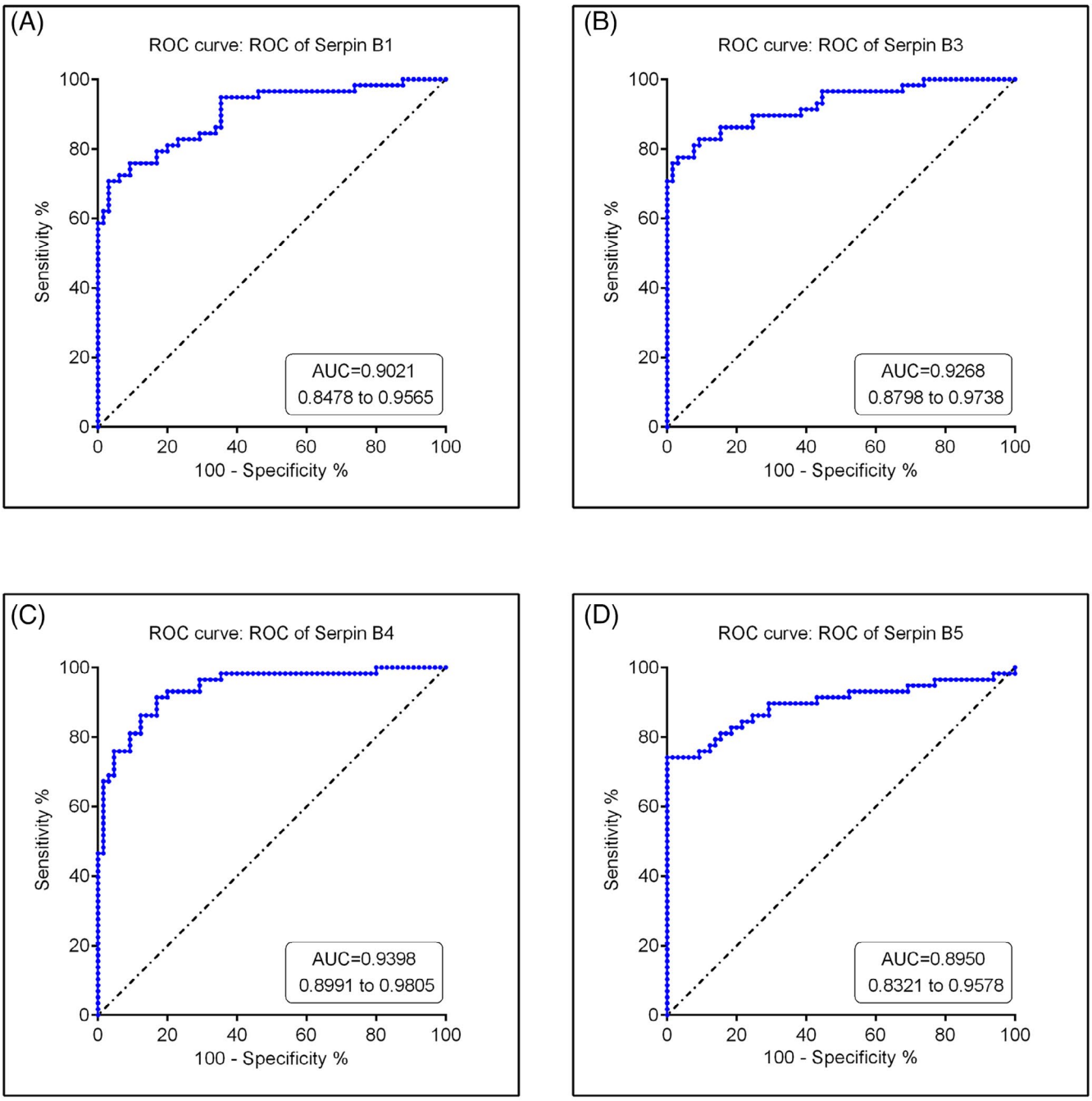

FIGURE 5 ROC curves of four SERPINBs' clinical significance in peri-implantitis. (A) SERPINB1, (B) SERPINB3 (C) SERPINB4 (D) SERPINB5 are analyzed by ROC curves and each SERPINBs represent the potential AUC

and TNF-α ($r = 0.3863$, $p < 0.0027$) (Figure 8C,D). Therefore, IL-6 and TNF-α were up-regulated expressed and directly correlated to four SERPINBs significantly in peri-implantitis patients with PICF.

4 | DISCUSSION

Currently, the accession of dental implants in the population has been incremented gradually together with the peri-implant-related diseases.[16] Where mostly peri-implantitis' precursor condition demonstrates as peri-implant mucositis. Therefore, the initiation of peri-implant mucositis's early diagnosis and treatment must be essential for peri-implantitis prevention.[17,18] Previous studies have demonstrated that nearly 45% of implant sites and closely 55% of implant patients could represent peri-implantitis disease,[6] which mostly shows damage in peri-implant tissue, specifically destruction of alveolar bone around implants and absolute inflammatory response, leads to loss of implant if left it without any treatment.[19]

SERPINBs are crucial in the function of the immune system and inflammation, and the production of mucous.[20] Previous researches

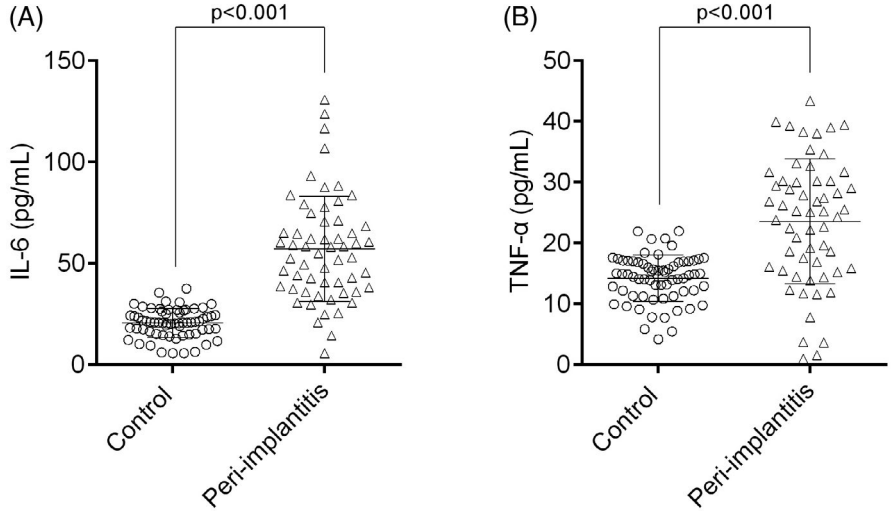

FIGURE 6 Expression of IL-6 and TNF-α between healthy and peri-implantitis patients in PICF. (A) IL-6 (B) TNF-α levels analyze by scatter plots

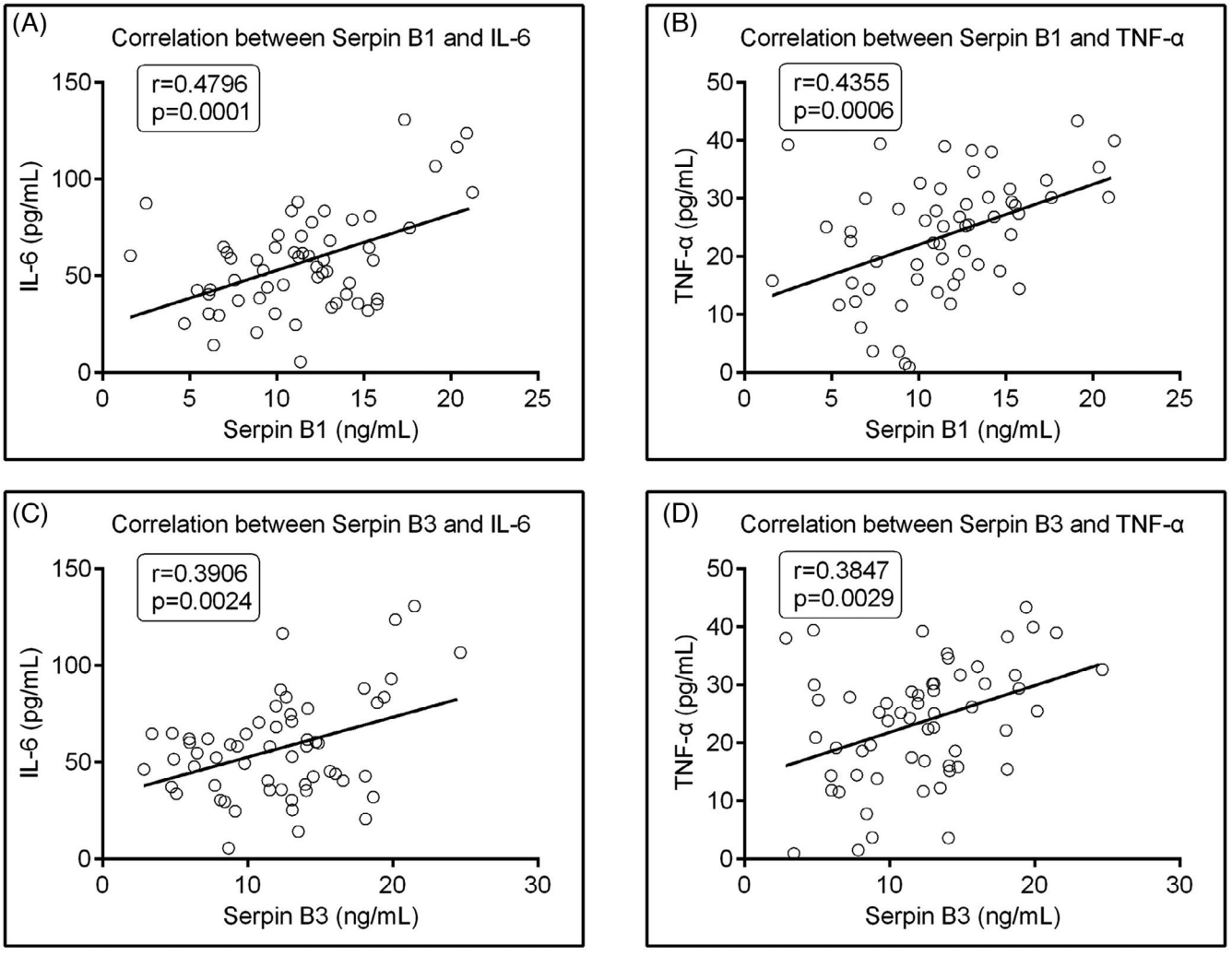

FIGURE 7 Correlation analysis of SERPINB1 and SERPINB3 to two cytokines in peri-implantitis. (A,B) SERPINB1 to IL-6 and TNF-α. (C,D) SERPINB3 to IL-6 and TNF-α were analyzed by Pearson's correlation

have reported that SERPINB1, B6, B7 and B9 are required in the function of the immune system combined with the development roles of neutrophil and megakaryocyte and the cytotoxic granule protease granzyme B inhibition.[21,22] Meanwhile, SERPINB3 and SERPINB4 are acted in the production of mucous and are expressed in the tissues of epithelial, for instance, tonsils, tongue, cervix, vagina, uterus, thymus, and in the upper respiratory tract.[23,24] Even though evasive function, SERPINB3 emerges role as in immunity and

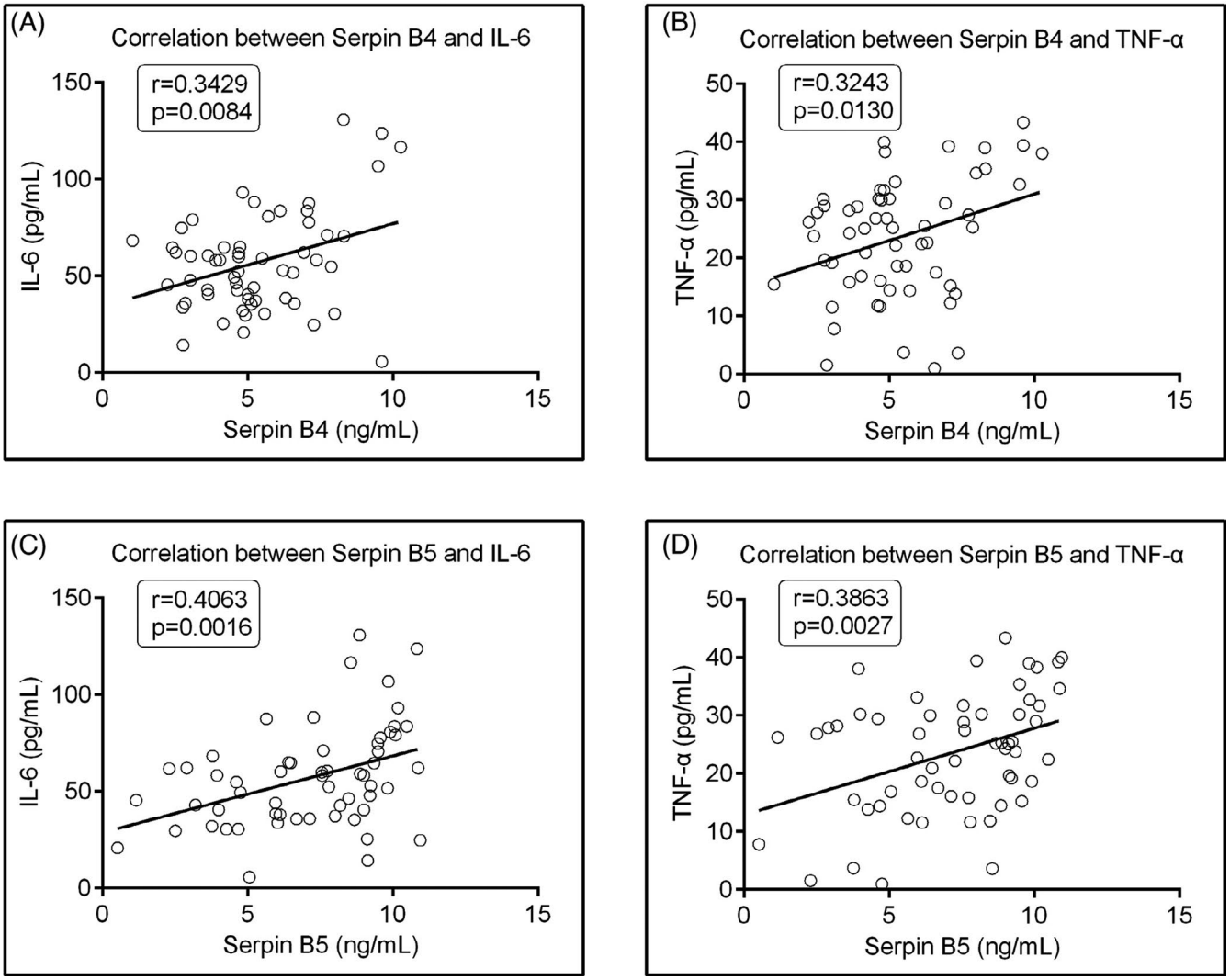

FIGURE 8 Correlation analysis of SERPINB4 and SERPINB5 to two cytokines in peri-implantitis. (A,B) SERPINB4 to IL-6 and TNF-α. (C,D) SERPINB5 to IL-6 and TNF-α were analyzed by Pearson's correlation

apoptotic regulation, which indicates SERPINB3 in the pathogenesis of autoimmunity and tumor-metastasis.[23] Besides, SERPINB5 had been reported in breast and prostate cancer as a tumor suppressor gene to inhibit metastasis.[23] Apart from that multiple SERPINBs, such as SERPINB12, B13, B4, B3, B7, B11, and B2, have been discovered in squamous cell carcinoma of oral.[3] The present study has discovered and validated the up-regulated expression of SERPINBs in peri-implantitis and healthy controls with PICF samples. Thus, on one hand, SERPINBs are representing significantly dysregulated expression in peri-implantitis with PICF and on the other hand, they may play role in the inflammatory pathogenesis of peri-implantitis. Furthermore, in the current study of four SERPINBs protein-coding genes were significantly distinguishing peri-implantitis and healthy controls of PICF samples with a potential AUC range from 0.895 to 0.939. Taken together, SERPINBs coding genes may not only affect the pathogenesis of peri-implantitis but also may accurately differentiate peri-implantitis from healthy controls.

Peri-implantitis is delineated by notable inflammation combined with peri-implant tissue destruction due to higher vascular permeability and blood flow in the impacted area, fluid exudation, migration, and blood vessels based collection of leukocytes to the tissue delineating peri-implant inflammation.[25,26] Meanwhile, PICF based SERPINBs expression was positively correlated to the PICF based on two different cytokines. Here, the PICF based two different cytokines (IL-6, TNF-α) showed up-regulated expression in peri-implantitis, which were consistent with previous studies.[27,28] Furthermore, previous findings reported that IL-6, IL-17, and IL-33 cytokines from PICF were escalating the process of local inflammation, incremental marginal bleeding and probing depth in peri-implantitis patients.[27,29] In this context, SERPINBs with cytokines may involve in the inflammatory process of development and progression of peri-implantitis disease.

Since the last decade, despite the dental implants in function for the first year, more than 2 mm bone loss around the dental implant indicates peri-implantitis in general[30-32] whereas not liner pattern of the bone loss has been reported previously.[33] Normally, the occurrence of peri-implantitis develops after passing the initial years of the in-function dental implant. Hence, considers that it is very

crucial to monitor the changes accurately which might develop in the initial phase of post-restorative dental implants accompanied by the attention of bleeding on suppuration or probing and in adjoined with bone loss by radiographic evidence. At present, for predicting the progression of peri-implantitis by prediction or algorithm models based on diagnostic methodology are not established or available for daily practice by clinical perspective. Therefore, further researches are needed for the development of the prediction model for peri-implantitis.

However, the present study has few limitations. Firstly, the discovery of four SERPINBs expressions was conducted by an online GEO-based database, which might represent biased samples or microarray findings. Secondly, the validation of four SERPINBs was analyzed with fewer samples, thus further studies need to validate it by a larger cohort. Thirdly, the two different cytokines were evaluated and compared using PICF samples without demonstrating clinical significance. Fourth, the current study was unable to explore SERPINBs in peri-implant mucositis patients and to compare with peri-implantitis. Therefore, further studies are needed to variously detect and evaluate SERPINBs with diverse cytokines, which may play role in the occurrence and progression of peri-implantitis.

5 | CONCLUSION

SERPINBs can peculiarly be detectable in peri-implantitis patients with PICF, which could act as up-regulated protein-coding genes that might play an influential role in causing peri-implantitis. SERPINBs may bound with other inflammatory cytokines in the pathogenesis of peri-implantitis.

CONFLICTS OF INTEREST

The authors declared that they have no potential conflicts of interest.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ORCID

Qingmei Wang https://orcid.org/0000-0002-3177-117X

REFERENCES

1. Yu T, Acharya A, Mattheos N, et al. Molecular mechanisms linking peri-implantitis and type 2 diabetes mellitus revealed by transcriptomic analysis. *PeerJ*. 2019;7:e7124.
2. Belibasakis GN. Microbiological and immuno-pathological aspects of peri-implant diseases. *Arch Oral Biol*. 2014;59:66.
3. Duarte PM, de Mendonca AC, Maximo MB, Santos VR, Bastos MF, Nociti Junior FH. Differential cytokine expressions affect the severity of peri-implant disease. *Clin Oral Implants Res*. 2009;20:514.
4. Kormas I, Pedercini C, Pedercini A, Raptopoulos M, Alassy H, Wolff LF. Peri-implant diseases: diagnosis, clinical, histological, microbiological characteristics and treatment strategies. A narrative review. *Antibiotics (Basel)*. 2020;9(11):835.
5. Academy of O. Guidelines of the Academy of Osseointegration for the provision of dental implants and associated patient care. *Int J Oral Maxillofac Implants*. 2010;25(620):2010.
6. Matys J, Botzenhart U, Gedrange T, Dominiak M. Thermodynamic effects after Diode and Er:YAG laser irradiation of grade IV and V titanium implants placed in bone - an ex vivo study. Preliminary report. *Biomed Tech (Berl)*. 2016;61:499.
7. Renvert S, Persson GR, Pirih FQ, Camargo PM. Peri-implant health, peri-implant mucositis, and peri-implantitis: case definitions and diagnostic considerations. *J Periodontol*. 2018;89 (Suppl 1):S304.
8. Barzkar N, Khan Z, Tamadoni Jahromi S, Pourmozaffar S, Gozari M, Nahavandi R. A critical review on marine serine protease and its inhibitors: A new wave of drugs? *Int J Biol Macromol*. 2021;170:674-687.
9. Kuo CS, Chen JS, Lin LY, et al. Inhibition of serine protease activity protects against high fat diet-induced inflammation and insulin resistance. *Sci Rep*. 2020;10(1):1725.
10. Szałapata K, Osińska-Jaroszuk M, Kapral-Piotrowska J, et al. Serine protease inhibitors-new molecules for modification of polymeric biomaterials. *Biomolecules*. 2020;10(1):82.
11. Irving JA, Steenbakkers PJ, Lesk AM, Op den Camp HJM, Pike RN, Whisstock JC. Serpins in prokaryotes. *Mol Biol Evol*. 2002;19:1881.
12. Atchley WR, Lokot T, Wollenberg K, Dress A, Ragg H. Phylogenetic analyses of amino acid variation in the serpin proteins. *Mol Biol Evol*. 2001;18:1502.
13. Mohammed ES, El-Dakhly KM. Serine protease inhibitor, serpin, is a biomarker following Schistosoma mansoni re-infection in mice. *Trop Biomed*. 2021;38:63.
14. Teke K, Kasap M, Simsek E, et al. SERPIN A5 may have a potential as a biomarker in reflecting the improvement of semen quality in infertile men who underwent varicocele repair. *Andrologia*. 2021;53(7):e14081.
15. Choi YJ, Kim S, Choi Y, et al. SERPINB1-mediated checkpoint of inflammatory caspase activation. *Nat Immunol*. 2019;20:276.
16. Daneshparvar H, Esfahanizadeh N, Vafadoost R. Dental implants in Sjögren syndrome. *Eur J Transl Myol*. 2020;30(2):8811.
17. Ata-Ali J, Flichy-Fernandez AJ, Alegre-Domingo T, Ata-Ali F, Palacio J, Penarrocha-Diago M. Clinical, microbiological, and immunological aspects of healthy versus peri-implantitis tissue in full arch reconstruction patients: a prospective cross-sectional study. *BMC Oral Health*. 2015;15:43.
18. Liu S, Li M, Yu J. Does chlorhexidine improve outcomes in non-surgical management of peri-implant mucositis or peri-implantitis?: A systematic review and meta-analysis. *Med Oral Patol Oral Cir Bucal*. 2020;25(5):e608-e615.
19. Li H, Chen Z, Zhong X, Li J, Li W. Mangiferin alleviates experimental peri-implantitis via suppressing interleukin-6 production and Toll-like receptor 2 signaling pathway. *J Orthop Surg Res*. 2019;14:325.
20. Vidalino L, Doria A, Quarta S, Zen M, Gatta A, Pontisso P. SERPINB3, apoptosis and autoimmunity. *Autoimmun Rev*. 2009;9:108.
21. Miyata T, Inagi R, Nangaku M, et al. Overexpression of the serpin megsin induces progressive mesangial cell proliferation and expansion. *J Clin Invest*. 2002;109:585.
22. Hou L, Yuki K. SerpinB1 expression in Th17 cells depends on hypoxia-inducible factor 1-alpha. *Int Immunopharmacol*. 2020;87:106826.
23. Gatto M, Iaccarino L, Ghirardello A, et al. Serpins, immunity and autoimmunity: old molecules, new functions. *Clin Rev Allergy Immunol*. 2013;45:267.
24. Sivaprasad U, Askew DJ, Ericksen MB, et al. A nonredundant role for mouse Serpinb3a in the induction of mucus production in asthma. *J Allergy Clin Immunol*. 2011;127:254.
25. Shiiba M, Nomura H, Shinozuka K, et al. Down-regulated expression of SERPIN genes located on chromosome 18q21 in oral squamous cell carcinomas. *Oncol Rep*. 2010;24:241.

26. Sánchez-Martos R, Samman A, Priami M, Arias-Herrera S. The diode laser as coadyuvant therapy in the non-surgical conventional treatment of peri-implant mucositis: a systematic review and meta-analysis. *J Clin Exp Dent*. 2020;12(12):e1171-e1182.
27. Yaghobee S, Khorsand A, Rasouli Ghohroudi AA, Sanjari K, Kadkhodazadeh M. Assessment of interleukin-1beta and interleukin-6 in the crevicular fluid around healthy implants, implants with peri-implantitis, and healthy teeth: a cross-sectional study. *J Korean Assoc Oral Maxillofac Surg*. 2014;40:220.
28. Liskmann S, Vihalemm T, Salum O, Zilmer K, Fischer K, Zilmer M. Correlations between clinical parameters and interleukin-6 and interleukin-10 levels in saliva from totally edentulous patients with peri-implant disease. *Int J Oral Maxillofac Implants*. 2006;21:543.
29. Severino VO, Beghini M, de Araujo MF, et al. Expression of IL-6, IL-10, IL-17 and IL-33 in the peri-implant crevicular fluid of patients with peri-implant mucositis and peri-implantitis. *Arch Oral Biol*. 2016;72:194.
30. Gholami H, Mericske-Stern R, Kessler-Liechti G, Katsoulis J. Radiographic bone level changes of implant-supported restorations in edentulous and partially dentate patients: 5-year results. *Int J Oral Maxillofac Implants*. 2014;29:898.
31. Tonetti M, Palmer R, Working Group 2 of the VEWoP. Clinical research in implant dentistry: study design, reporting and outcome measurements: consensus report of Working Group 2 of the VIII European Workshop on Periodontology. *J Clin Periodontol*. 2012;39 (Suppl. 12):73.
32. Heyman O, Horev Y, Koren N, et al. Niche specific microbiota-dependent and independent bone loss around dental implants and teeth. *J Dent Res*. 2020;99(9):1092-1101.
33. Derks J, Schaller D, Hakansson J, Wennstrom JL, Tomasi C, Berglundh T. Effectiveness of implant therapy analyzed in a swedish population: prevalence of peri-implantitis. *J Dent Res*. 2016;95:43.

How to cite this article: Jiang J, Gao G, Wang Q. Levels of SERPIN family proteins in peri-implant crevicular fluid in patients with peri-implantitis. *J Clin Lab Anal*. 2021;35:e23926. https://doi.org/10.1002/jcla.23926