Expression of HORMAD1 in Chronic Rhinosinusitis and Its Correlation with Inflammatory Factors

Xin Zhang, Yu Xu and Jinhui Zhou

Department of Otolaryngology Head and Neck Surgery, The Affiliated Huai'an No.1 People's Hospital of Nanjing Medical University, Huai'an, China

ABSTRACT

Objective: To explore the abnormal expression of HORMA domain containing protein 1 (HORMAD1) in chronic rhinosinusitis (CRS) patients and its correlation with the expression of inflammatory factors.

Study Design: Observational study.

Place and Duration of the Study: Department of Otolaryngology Head and Neck Surgery, the Affiliated Huai'an No.1 People's Hospital of Nanjing Medical University, China, from August 2021 to October 2022.

Methodology: Eighty CRS patients and 80 healthy volunteers participated in this study according to the inclusion and exclusion criteria. The plasma samples of the patients were collected and the level of HORMAD1 was determined by RT-qPCR methods. Commercially available ELISA kits were used for the detection of the levels of cytokines, and the correlation between HORMAD1 expression and the level of cytokines was analysed.

Results: HORMAD1 expression was significantly increased in CRS patients as compared to the healthy subjects. Moreover, the results of ROC curve suggested AUC for HORMAD1 was 0.9442, 95% confidence interval, 0.9057 to 0.9827. IL-1β, TNF-α, IL-6 as well as IFN-γ were all markedly elevated in the CRS group. Positive correlations were found between HORMAD1 expression and the cytokines.

Conclusion: HORMAD1 may trigger an inflammatory response in CRS patients. The results of the current study could be beneficial for improving the therapeutic efficacy of CRS patients.

Key Words: *HORMAD1, Chronic rhinosinusitis, Expression, Inflammatory factors, DNA damage, Cytokines.*

How to cite this article: Zhang X, Xu Y, Zhou J. Expression of HORMAD1 in Chronic Rhinosinusitis and Its Correlation with Inflammatory Factors. *J Coll Physicians Surg Pak* 2024; **34(01)**:54-57.

INTRODUCTION

Rhinosinusitis is a common otorhinolaryngological disease. If inflammation and symptoms persist for more than 12 weeks, it is named chronic rhinosinusitis (CRS).[1] CRS is not only one of the most common upper respiratory tract diseases in the Western countries but also widely increasing in the Asian regions.[2] The epidemiological data demonstrated that the prevalence of CRS was estimated to be more than 8% in China according to the nasal polyps diagnostic criteria.[3] The prevalence of asthma in CRS patients is more than 2 times higher than in non-CRS patients, which brings great distress to their work and life. A prior research found that CRS caused a 36% reduction in patients' productivity.[4]

Correspondence to: Dr. Xin Zhang, Department of Otolaryngology Head and Neck Surgery, The Affiliated Huai'an No.1 People's Hospital of Nanjing Medical University, Huai'an, China
E-mail: drzhangxin1109@outlook.com

Received: January 09, 2023; Revised: November 08, 2023; Accepted: December 21, 2023
DOI: https://doi.org/10.29271/jcpsp.2024.01.54

Due to the high heterogeneity of CRS, the key links causing CRS disease are still not very clear, resulting in unsatisfactory efficacy and refractory clinical characteristics, becoming an important problem plaguing doctors and patients.[5] The previous studies found changes in the inflammatory pattern of CRS patients in China; furthermore, in the past 20 years, the comorbidities of CRS and asthma had significantly increased seriously affecting people's production and life.[6] If timely and effective treatment is not taken, the symptoms of respiratory infections in patients with CRS will aggravate. If the symptoms are severe, it will also affect important organs such as skull, eyes, and lungs.[7] Early diagnosis and timely intervention are of great significance for the progression of the disease in CRS.

HORMA domain containing protein 1 (HORMAD1) is a member of the cancer/testis antigens (CTAs) family, which may cause immunogenicity to induce spontaneous antibody responses in patients' bodies.[8] The HORMA domain proteins family is known to regulate the cell cycle and plays a key role in mitosis and meiosis.[9,10] HORMAD1 is expressed during gonadal development and participates in the progress of meiosis. HORMAD1 is upstream of ataxia telangiectasia mutated (ATM) kinase activation, a serine-threonine protein kinase, and the absence of HORMAD1 interferes with the autophosphorylation of ATMs.[9] The reduction of ATM phosphorylation is thought to be related to the cell cycle, apoptosis, and response to DNA damage.[11]

Numerous researches reported that HORMAD1 may be involved in the progression in different diseases.[12,13] Nevertheless, there had been no studies reporting the role of HORMAD1 in patients with CRS and its function is also unclear. In order to explore the mechanism of CRS, gene sequencing has been conducted and the results revealed differential expression of HORMAD1 in tissue samples of CRS patients and healthy people.

The objective of this study was to determine the expression of HORMAD1 in CRS was detected by bioinformatics and PCR, and analyse the relationship between HORMAD1 and the inflammatory factors of patients.

METHODOLOGY

Eighty CRS patients who were hospitalised at the Affiliated Huai'an No.1 People's Hospital of Nanjing Medical University between August 2021 and October 2022 were collected. The tissue samples and peripheral blood samples for 80 CRS patients and 80 healthy volunteers were extracted to detect. An ethical approval and informed patients' consents were obtained. The inclusion criteria was subjects diagnosed with CRS based on the European EAACI Position Paper on Rhinosinusitis and Nasal Polyps published in 2012.[14] The exclusion criteria was subjects who had severe complications of nasal eye and nasal skull base, and severe systemic diseases. At the same time, gestational patients were also excluded from this experiment.

Five ml of preoperative peripheral blood samples of patients in the sinusitis group and control group were taken and the serum was separated by centrifugation and stored at -80°C, the serum specimen was taken, and the content of IL-1β, TNF-α, IL-6, and IFN-γ were determined by enzyme-linked immunosorbent assay (ELISA) kit.

RT-PCR was performed according to invitrogen's Trizol and reverse transcription kits and TAKARA's SYBR Green kit instructions. The primers for the PCR reaction were synthesised by Inwei Jieji (Shanghai) Co., Ltd. The reaction conditions were 95°C for 160 seconds; 40 cycles of 95°C for 15 seconds, 60°C for 15 seconds and 72°C for 45 seconds; 95°C for 15 seconds and 60°C for 15 seconds. Correlation statistical analysis was performed based on the CT value of each well.

All statistical analysis has been completed by Graph Pad. The data were expressed as mean ± standard deviation, and Student's t-test was conducted for comparison between the groups. The categorical indicators described the number of cases and percentages of each type. $p < 0.05$ was considered statistically significant. Area under ROC Curve (AUC) had been conducted for evaluating diagnostic value of HORMAD1 in samples. And Pearson's correlation coefficient was employed to analyse the correlation between the variables.

RESULTS

Figure 1A shows the differentially expressed genes in tissue samples of CRS patients, among them, HORMAD1 demonstrated high expression. The sequence detection extracted 80 samples of CRS patients and healthy subjects, with a total of 357 differential genes, including 191 upregulated genes and 166 downregulated genes, of which HORMAD1 belongs to the upregulated gene (Figure 1B).

The comparison results of HORMAD1 expression level between the two groups are shown in Figure 2A. HORMAD1 expression levels were significantly increased in CRS patients as compared to the healthy subjects. Moreover, the results of ROC analysis indicated AUC for HORMAD1 was 0.9442, 95% confidence interval, 0.9057 to 0.9827 (Figure 2B).

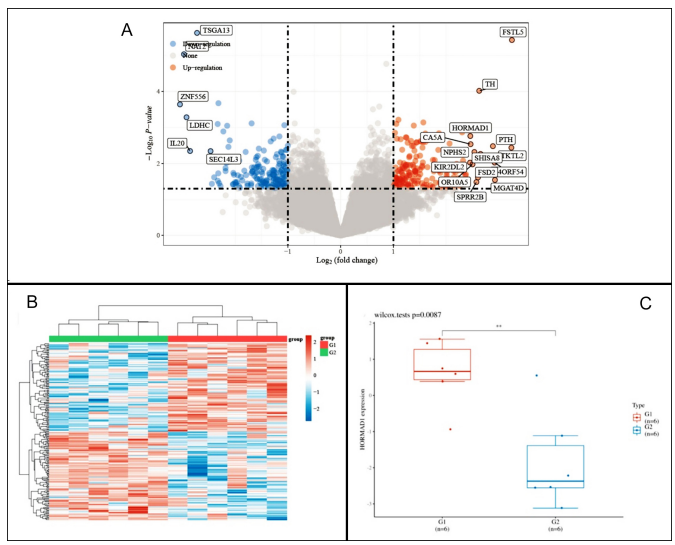

Figure 1: Differentially expressed genes in CRS. (A) Differentially expressed genes in tissue samples of CRS patients are showed using the volcano plots. (B) Differentially expressed genes are exhibited using the heat map. Red refers to up-regulated genes, and blue refers to down-regulated genes. (C) The expression of HORMAD1 was analysed in tumour tissues (G1; n=6) and tumour adjacent tissues (G2; n=18).

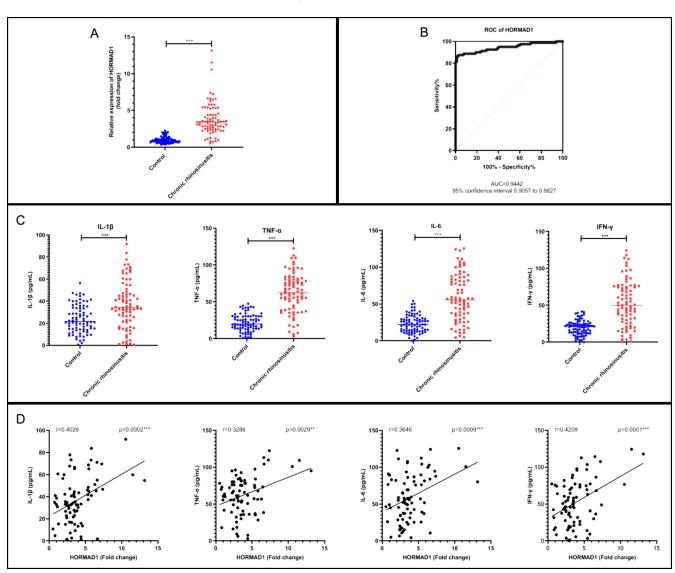

Figure 2: HORMAD1 expression levels were significantly increased in CRS patients. (A) The comparison results of HORMAD1 expression level between two groups. (B) ROC analysis. (C) Elevated expression of inflammatory factors in peripheral blood in patients with CRS. (D) Correlation between the level of HORMAD1 and cytokines in patients with CRS.

Expressions of inflammatory factors were detected and compared between the CRS patients and healthy subjects. The results showed in Figure 2C that IL-1β, TNF-α, IL-6 as well as IFN-γ were all markedly elevated in the CRS group.

As shown in Figure 2D, the correlation between HORMAD1 and the level of cytokines were explored. Significantly positive correlations were found between HORMAD1 expression and the levels of IL-1β (r=0.4026, p=0.0002***), TNF-α (r=0.3286, p=0.0029**), IL-6 (r=0.3646, p=0.0009**) as well as IFN-γ (r=0.4209, p=0.0001***).

DISCUSSION

The previous studies had shown that HORMAD1 was upregulated in various cancers, which was highly consistent with the current findings. Bian *et al.* believed that HORMAD1 plays an important role in gastric cancer progression and could be a promising prognostic biomarker and therapeutic target.[15] Rania *et al.* indicated that HORMAD1 was over-expressed in 71% of TNBC.[16] In the current study, the HORMAD1 expression level was tested in CRS and healthy groups, and the potential connection between inflammatory response was analysed in CRS patients and HORMAD1 expression. The final results of correlation analysis displayed that HORMAD1 expression in peripheral blood of patients with sinusitis was positively associated with IL-1β, TNF-α, IL-6 as well as IFN-γ expressions.

A prior study published by Luo *et al*. investigated the changes of inflammatory pattern of CRS in Asian areas and mentioned inflammatory response in CRS patients.[17] Inflammation is the basic pathological process of many diseases, which can occur in tissues and organs in various parts of the body.[18] The sustained chronic inflammatory response of CRS can lead to the proliferation of nasal mucosal goblet cells, increased mucus secretion, and obstruction of drainage disorders. On the basis of this, it will cause the mucus in the sinus cavity to be unable to drain and further stimulate the activation of inflammatory response cascade expansion and the secretion of purulent secretions in the nasal mucosa.[19]

This study found that the levels of IL-1β, TNF-α, IL-6, IFN-γ in peripheral blood samples of CRS patients were significantly increased. IL-1β, TNF-α, IL-6 as well as IFN-γ were all known as pro-inflammatory cytokines, which were synthesised primarily from Th1 cells, mediated the cellular immune response and was capable of causing cascade amplification of inflammatory responses in the local mucosal tissues.[20] This data indicated the expression of HORMAD1 was strongly associated with IL-1β, TNF-α, IL-6, and IFN-γ expressions in the peripheral blood of CRS patients. The role of HORMAD1 in chronic inflammatory response in CRS is a question-worth exploring. The accumulating evidences suggested that HORMAD1 is over-expressed in cancerous tissues with a variety of cancers. To investigate the mechanism of aberrantly expressed HORMAD1 in tumour cells, Liu *et al*.[21] undertook a study and discovered that abnormally expressed HORMAD1 in cancer cells causes DNA damage and disrupts DNA mismatch repair by blocking effective nuclear localization. A recent study conducted by Ragu *et al.*[22] also suggested that DNA damage is usually observed in the early or precancerous stages of cancer, which is consistent with Liu's results. Moreover, it had been reported that DNA damage can lead to defects in DNA repair, resulting in weakness in DNA function, as well as induction of cytokines and lead to auto-inflammatory diseases.[23] Francis *et al*. showed that damaged human cells develop persistent chromatin lesions bearing hallmarks of DNA double-strand breaks (DSBs), which initiate increased secretion of inflammatory cytokines such as interleukin-6 (IL-6).[24] Shane *et al.* observed a delayed accumulation of active STAT1 and inflammatory gene expression, confirming these signals are driven by DSBs.[25]

The authors proposed the hypothesis that HORMAD1 causes DNA damage, which triggers an inflammatory response, while the pathological nature of chronic sinusitis is a chronic inflammatory response, so the expression of HORMAD1 in CRS patients is positively correlated with levels of pro-inflammatory factors, such as IL-1β, TNF-α, IL-6, and IFN-γ. However, more in-depth researches should be conducted in the future for validating this hypothesis. There are still some other limitations. The present study included only 80 CRS patients and 80 healthy people who voluntarily participated in the study which may cause some recruitment bias. In addition, the included samples were relatively small, which may reduce the statistical power. Further validation of this effect through well-designed clinical trials will be necessary in the future.

CONCLUSION

HORMAD1 expression levels were significantly increased in CRS patients and were associated with the levels of pro-inflammatory cytokines. HORMAD1 may trigger an inflammatory response in CRS patients by causing DNA damage. The results of the current study indicated that HORMAD1 may be one of the important indicators in the early process of CRS patient diagnosis, and could be beneficial for improving the therapeutic efficacy of CRS patients.

ETHICAL APPROVAL:

The study was approved by the Ethics Committee of Affiliated Huai'an No.1 People's Hospital of Nanjing Medical University, China on 6/5/2021.

PATIENTS' CONSENT:

Informed consents were obtained from all the patients.

COMPETING OF INTEREST:

The authors reported no conflict of interest in regard to this work.

AUTHORS' CONTRIBUTION:

XZ: Conceived the idea of the study.
JZ: Performed the data analysis and wrote the manuscript.
YX: Analysed the data and prepared the manuscript.
All authors approved the final version of the manuscript to be published.

REFERENCES

1. Hirsch AG, Stewart WF, Sundaresan AS, Young AJ, Kennedy TL, Scott Greene J, *et al.* Nasal and sinus symptoms and chronic rhinosinusitis in a population-based sample. *Allergy* 2017; **72(2)**:274-81. doi: 10.1111/all.13042.
2. Zhang Y, Gevaert E, Lou H, Wang X, Zhang L, Bachert C, *et al.* Chronic rhinosinusitis in Asia. J *Allergy Clin Immunol* 2017; **140(5)**:1230-9. doi: 10.1016/j.jaci.2017.09.009.
3. Shi JB, Fu QL, Zhang H, Cheng L, Wang YJ, Zhu DD, *et al*. Epidemiology of chronic rhinosinusitis: Results from a cross-sectional survey in seven Chinese cities. *Allergy* 2015; **70(5)**:533-9. doi: 10.1111/all.12577.
4. Wang Y, Ghoshal AG, Bin Abdul Muttalif AR, Lin HC, Thanaviratananich S, Bagga S, *et al.* Quality of life and economic burden of respiratory disease in Asia-Pacific-Asia-Pacific burden of respiratory diseases study. *Value Health Reg Issues* 2016; **9**:72-7. doi: 10.1016/j.vhri.2015.11.004.
5. Zhang Y, Zhang L. Increasing prevalence of allergic rhinitis in China. *Allergy, Asthma Immunolo Res* 2019; **11(2)**:156. doi: 10.4168/aair.2019.11.2.156.
6. Lin J, Wang W, Chen P. Prevalence and risk factors of asthma in mainland China: The CARE study. *Respir Med* 2018; **137**:48-54. doi: 10.1016/j.rmed.2018.02.010.
7. Ghogomu N, Kern R. Chronic rhinosinusitis: The rationale for current treatments. *Expert Rev Clin Immunol* 2017; **13(3)**:259-70. doi: 10.1080/1744666X.2016.1220833.
8. Chen Y-T, Venditti CA, Theile G. Identification of CT46 HORMAD1, an immunogenic cancer testis antigen encoding a putative meiosis-related protein. *Cancer Immun* 2005; **5**:9.
9. Shin YH, Choi Y, Erdin SU. HORMAD1 mutation disrupts synaptonemal complex formation, recombination, and chromosome segregation in mammalian meiosis. *PLoS Genetics* 2010; **6(11)**:e1001190. doi: 10.1371/journal.pgen.1001190.
10. Aravind L, Koonin EV. The HORMA domain: A common structural denominator in mitotic checkpoints, chromosome synapsis and DNA repair. *Trends Biochem Sci* 1998; **23(8)**:284-6. doi: 10.1016/s0968-0004(98)01257-2.
11. Weitering TJ, Takada S, Weemaes CMR, van Schouwenburg PA, van der Burg M. ATM: Translating the DNA damage response to adaptive immunity. *Trends Immunol* 2021; **42(4)**:350-65. doi: 10.1016/j.it.2021.02.001.
12. Chen B, Tang H, Chen X. Transcriptomic analyses identify key differentially expressed genes and clinical outcomes between triple-negative and non-triple-negative breast cancer. *Cancer Manag Res* 2019; **11**:179-90. doi: 10.2147/CMAR.S187151.
13. Wang X, Tan Y, Cao X. Epigenetic activation of HORMAD1 in basal-like breast cancer: Role in Rucaparib sensitivity. *Oncotarget* 2018; **9(53)**: 30115-27. doi: 10.18632/oncotarget.25728.
14. Fokkens WJ, Lund VJ, Mullol J, Bachert C, Alobid I, Baroody F, *et al*. European position paper on rhinosinusitis and nasal polyps 2012. A summary for otorhinolaryngologists. *Rhinology* 2012; **50(1)**:1-12. doi: 10.4193/Rhino12.000.
15. Bian G, Li W, Huang D. The cancer/testis antigen HORMAD1 promotes gastric cancer progression by activating the NF-κB signaling pathway and inducing epithelial-mesenchymal transition. *Am J Transl Res* 2023; **15(9)**:5808-25.
16. El-Botty R, Vacher S, Mainguené J. HORMAD1 overexpression predicts response to anthracycline-cyclo-phosphamide and survival in triple-negative breast cancers. *Mol Oncol* 2023; **17(10)**:2017-28. doi: 10.1002/ 1878-0261.13412.
17. Luo X, Xu Z, Zuo K, Deng J, Gao W, Jiang L. The changes of clinical and histological characteristics of chronic rhinosinusitis in 18 years: Was there an inflammatory pattern shift in southern China? *World Allergy Organ J* 2021; **14(4)**:100531. doi: 10.1016/j.waojou.2021.100531.
18. Medzhitov R. Origin and physiological roles of inflammation. *Nature* 2008; **454(7203)**:428-35. doi: 10.1038/nature07201.
19. Shin SH, Kim YH, Ye MK, Choi SY. Immunopathologic characteristics of nasal polyps in adult Koreans: A single-center study. *Am J Rhinol Allergy* 2017; **31(3)**:168-73. doi:10.2500/ajra.2017.31.4423.
20. Gao Y, Mutter-Rottmayer E, Greenwalt A. A neomorphic cancer cell-specific role of MAGE-A4 in trans-lesion synthesis. *Nature Communications* 2016; **7**:12105. doi: 10.1038/ncomms12105.
21. Liu K, Wang Y, Zhu Q, Li P, Chen J, Tang Z. Aberrantly expressed HORMAD1 disrupts nuclear localization of MCM8-MCM9 complex and compromises DNA mismatch repair in cancer cells. *Cell Death Dis* 2020; **11(7)**:519. doi: 10.1038/s41419-020-2736-1.
22. Ragu S, Matos-Rodrigues G, Lopez BS. Replication Stress, DNA Damage, Inflammatory Cytokines and Innate Immune Response. *Genes (Basel)* 2020; **11(4)**:409. doi: 10.3390/genes11040409.
23. Li T Chen ZJ. The cGAS-cGAMP-STING pathway connects DNA damage to inflammation, senescence, and cancer. *J Exp Med* 2018; **215(5)**:1287-99. doi:10.1084/jem.20180139.
24. Rodier F, Coppe JP, Patil CK. Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. *Nat Cell Biol* 2009; **11(10)**:1272. doi:10.1038/ncb1909.
25. Harding, SM, Benci JL, Irianto J, Discher DE, Minn AJ, Greenberg RA. Mitotic progression following DNA damage enables pattern recognition within micronuclei. *Nature* 2017; **548(7668)**:466-70. doi: 10.1038/nature23470.