

Human Beta-Defensin 2 in Children with IgA Vasculitis: A Potential Link Between Innate Immunity and Vascular Inflammation

Dorukcan Alkan

dorukcanalkan@gmail.com

Etlik City Hospital

Ramazan Berk Us

Etlik City Hospital

Emine Nur Sunar Yayla

Etlik City Hospital

Aysenur Alkaya

Etlik City Hospital

Abdulahap Sen

Etlik City Hospital

Bahar Demirbas

Etlik City Hospital

Tuba Gundogdu

Etlik City Hospital

Ceren Dinler Yarasir

Etlik City Hospital

Nesibe Gokce Kocamaz

Etlik City Hospital

Sanem Sert

Etlik City Hospital

Ali Kansu Tehci

Etlik City Hospital

Elif Arslanoglu Aydin

Etlik City Hospital

Pakize Nilufer Akpinar Tekgoz

Etlik City Hospital

Esra Baglan

Etlik City Hospital

Semanur Ozdel

Research Article

Keywords: IgA vasculitis, beta-defensins

Posted Date: March 8th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-8883108/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Immunoglobulin A vasculitis is the most common systemic vasculitis in children, typically presenting with palpable purpura along with gastrointestinal, musculoskeletal, or genitourinary involvement. Human Beta Defensin-2, is the first inducible antimicrobial peptide identified in humans and is mainly synthesized by epithelial cells of the skin, lungs, and gastrointestinal tract and its levels increase in inflammation. Because Human Beta Defensin-2 is an inflammation-induced peptide, we aimed to investigate its potential role in immunoglobulin A vasculitis. Serum Human Beta Defensin-2 levels were measured using a ELISA kit according to the manufacturer's protocol. Serum Human Beta Defensin-2 levels were significantly higher in the immunoglobulin A vasculitis group than healthy controls ($p = 0.005$). Among patients with immunoglobulin A vasculitis, Human Beta Defensin-2 levels were higher in those who are 10 years or older ($p = 0.008$), with normal acute phase reactant levels ($p = 0.015$), and higher in those who had spontaneous disease onset ($p = 0.015$). This study indicated that serum Human Beta Defensin-2 levels were significantly higher in children with IgA vasculitis compared to healthy controls. Considering being an inflammation-triggered molecule, it's interesting that human beta defensin-2 levels were higher in patients with normal acute phase reactant levels, That elevation pattern suggests that Human Beta Defensin-2 may reflect a disease-specific immune response and could serve as a complementary biomarker in immunoglobulin A vasculitis.

Introduction

Immunoglobulin A vasculitis (IgAV) is the most common systemic vasculitis in children, typically presenting with palpable purpura along with gastrointestinal, musculoskeletal, or genitourinary involvement ¹. It is a leukocytoclastic vasculitis characterized by IgA-dominant immune complex deposition in small vessels. Renal findings resemble IgA nephropathy, with mesangial IgA-containing deposits leading to hematuria and proteinuria, whereas systemic features are attributed to IgA mediated endothelial injury and neutrophil driven inflammation. Complement components such as factor B, C3, C4d, and C5b-9 frequently accumulate in skin and kidney biopsies during the acute phase, accompanied by reduced serum complement levels, supporting activation of the lectin and alternative pathways ².

Human Beta Defensin-2 (hBD-2), discovered in 1997, is the first inducible antimicrobial peptide identified in humans ³. β -defensins, found only in vertebrates, form a distinct family based on their conserved six-cysteine structure and sequence pattern ⁴. hBD-2 is mainly synthesized by epithelial cells of the skin ³, lungs ⁵, and gastrointestinal tract ⁶. It is induced by activator protein 1 (AP-1), nuclear factor kappa B (NF- κ B) ⁷, tumor necrosis factor alpha (TNF- α) ⁸, interleukin 1 beta (IL-1 β)⁹, and lipopolysaccharide^{10 11}. It binds C1Q and inhibits the classical complement pathway without affecting the alternative pathway ¹². hBD-2 provides antimicrobial activity against *Candida* species and Gram-negative bacteria (3,4), functions as a chemotactic factor for neutrophils, T cells, and dendritic cells via C-C chemokine receptor 6 (CCR6) (^{13,14}), and protects mucosal and epithelial surfaces continuously exposed to microorganisms (^{15,16}). Its levels increase in acute infections and inflammatory diseases such as psoriasis ¹⁷, cystic

fibrosis¹⁸, and inflammatory bowel disease¹⁹. Due to its antimicrobial and immunomodulatory effects, hBD-2 also has therapeutic potential²⁰.

Given that hBD-2 is an inflammation-induced peptide, we aimed to investigate its potential role in IgAV. Our primary objective was to compare hBD-2 levels in children with IgAV and healthy controls. The secondary objective was to evaluate whether hBD-2 levels differ between IgAV patients with gastrointestinal and/or genitourinary involvement and those with only skin and joint involvement.

Materials & Methods

Patient Selection and Laboratory Investigations:

We enrolled 45 patients with IgAV who applied to our pediatric rheumatology clinic between 11/2024 and 05/2025 and 35 healthy controls who also were consulted to our clinic for rheumatologic diseases but excluded for rheumatologic diseases. Their human beta defensin-2 levels were compared. Patients were also divided into two groups each by joint involvement, abdominal involvement, high acute phase reactants, trigger factors associated with IgAV and MEFV genes if performed and studied.

Phlebotomy for human beta defensin-2 was done before steroid use if used in IgAV patients. Healthy controls had no infections or antibiotic or steroid usage in the past week. Testing for hBD-2 usually had been done simultaneously with other tests (i.e. complete blood count, acute phase reactants and MEFV gene test).

Blood samples were collected from the patient, accompanied by the patient's informed consent form, in two separating gel biochemistry equipment. They were left in the room for 20 minutes to break up clots. The tubes were then spun at 1500×g for 10 minutes. After centrifugation, serum aliquots were transferred to capped microvolume tubes and stored at -80°C until used. On the day of the study, the serum was stored at -80°C and brought to room temperature to thaw. Thawed samples were vortexed to homogenize the samples, and biochemical parameter observations were initiated. This study approved by the Ethic Committee of Etlik City Hospital in 12/18/2024 (Number of Adjudication: AESH-BADEK-2024-1229).

Beta Defensin-2 Level Determination

Beta Defensin-2 levels in serum samples were determined using an enzyme-linked immunosorbent assay (ELISA) kit (Elabscience, Cat No: E-EL-H0996, Houston, Texas) and the manufacturer's recommended certificate. The following steps are performed:

Beta Defensin-2 standards are prepared according to the kit's intended use. Standards and samples were added to the wells as 100 µL. The plate was incubated for 90 minutes in a 37°C incubator (Heidolph Incubator 1000, Germany). After incubation, the plate was washed 3 times with wash buffer using a plate

washer (BioTek, Winooski, USA). Residual wash buffer remaining in the wells was thoroughly removed. Then, 100 µL of streptavidin-HRP solution was added. The plate was sealed with foil and incubated for 30 minutes in a 37°C incubator (Heidolph Incubator 1000, Germany). After incubation, the plate was washed five times with wash buffer using a plate washer (BioTek, Winooski, USA). 50 µL of the solution was added to the final well, and the samples were observed to turn yellow. Sample and standard absorbances were read at 450 nm on a microplate reader spectrophotometer (BioTek, Winooski, USA).

A standard curve is generated using the absorbance values obtained against the standard distributions. Beta-defensin-2 levels in the samples were calculated using this standard curve, and the results are expressed in pg/dL.

Results

Our study included 45 children with IgAV (40% male; median age, 107 months; 24–202 months) and 35 healthy controls (43% male; median age, 144 months; 10–214 months).

Prior to the onset of IgAV, 8 patients (18.2%) experienced spontaneous disease onset, while 23 (52.3%) had a preceding upper respiratory tract infection, 5 (11.4%) had acute gastroenteritis, 3 (6.8%) had lymphadenitis, 3 (6.8%) experienced trauma, 1 (2.3%) had a skin infection, and 1 (2.3%) had preseptal cellulitis. All patients presented with rash. In addition, 19 patients (42.2%) reported abdominal pain, 21 (46.7%) had arthritis, 12 (26.7%) had subcutaneous edema, and 1 (0.5%) had scrotal or testicular pain.

Abdominal ultrasonography was performed in 26 patients (57.8%), and 7 of them (26.9%) showed findings suggestive of gastrointestinal involvement. Steroid therapy was administered to 19 patients (42.2%) for a mean duration of 29 days (range, 10–47 days), and 4 (21%) of these patients received pulse steroids. Among steroid-treated patients, corticosteroids were used for refractory skin lesions in 3 cases, gastrointestinal involvement in 14 cases, combined skin and gastrointestinal involvement in 1 case, and combined gastrointestinal and scrotal involvement in 1 case.

Elevated acute phase reactants (erythrocyte sedimentation rate and/or C-reactive protein) were detected in 32 patients (71.7%). MEFV gene analysis was performed in 21 patients, and 10 (47.6%) had at least one mutation, 7 of which (70%) were located in exon 10 (Table 1).

The median interval between symptom onset and hBD-2 measurement was 3 days (range, 0–7 days). Serum hBD-2 levels were significantly higher in the IgAV group (median 10.410 pg/dL; 6.410–55.614 pg/dL) compared with healthy controls (median 8.662 pg/dL; 5.438–18.303 pg/dL) ($p = 0.005$, Fig. 1). Among IgAV patients, hBD-2 levels were higher in those aged 10 years or older ($p = 0.008$, Fig. 2), higher in patients with normal acute phase reactant levels ($p = 0.015$, Fig. 3), and lower in patients with a preceding upper respiratory tract infection compared with those with spontaneous disease onset ($p = 0.015$, Fig. 4).

Discussion

IgA vasculitis is the most common systemic vasculitis in childhood, and a significant proportion of patients report a preceding infection, with upper respiratory tract infections being the most frequent trigger²¹. In our study, the presence of an upper respiratory tract infection in more than half of the patients supports this well established association. However, the fact that approximately one-fifth of the cases had a spontaneous onset suggests that, beyond infection, host immune responses and genetic predisposition may also play a role in the pathogenesis of IgAV.

One of the main findings of our study was the significantly higher serum hBD-2 levels in patients with IgAV compared to healthy controls. This result suggests that hBD-2 may be induced not only by infectious stimuli but also by the inflammatory and immunomodulatory mechanisms driven by the vasculitic process itself. hBD-2 is known to be synthesized in epithelial tissues and possesses both immunoregulatory and tissue-repairing properties (^{12,20}). Previous studies have reported increased hBD-2 expression in the terminal ileum in inflammatory bowel disease (¹⁹) and its potential value as a biomarker for the diagnosis and monitoring of ulcerative colitis (²²). Nevertheless, the lack of association between serum hBD-2 levels and gastrointestinal involvement in our study strengthens the possibility that hBD-2 may remain locally synthesized in tissues and may not be sufficiently released into the bloodstream. Therefore, hBD-2 measurements in other biological samples, such as stool or urine, may provide more meaningful information regarding organ-specific involvement.

Another noteworthy finding was the markedly higher hBD-2 levels in IgAV patients older than 10 years. The absence of this age-related difference in the control group suggests that the increase in hBD-2 cannot be explained solely by age-related physiological changes and may represent a disease specific immune response. This may be related to age-dependent differences in immune maturation or variations in the immunological phenotype of the disease; however, this observation is not clearly supported by current literature²³.

In our study, the lower hBD-2 levels in patients with a history of upper respiratory tract infection compared to those with spontaneous disease onset appear, at first glance, to contradict previous reports that hBD-2 increases during infections²⁴. This finding suggests that the elevation of hBD-2 in IgAV may be linked to vasculitic inflammation rather than infection itself. Similarly, higher hBD-2 levels in patients with normal acute phase reactants indicate that hBD-2 may be regulated independently of classical inflammatory markers such as ESR and CRP. In our study, complete blood count parameters and acute phase reactants reflected an inflammatory process consistent with the literature²⁵. Thus, hBD-2 may represent a complementary biomarker reflecting a different aspect of inflammation in IgAV.

MEFV mutations have been suggested to influence the clinical course of IgAV, with exon 10 mutations reportedly associated with more severe outcomes, including gastrointestinal involvement (^{26,27}). However, the lack of difference in hBD-2 levels according to MEFV mutation status in our study suggests that this relationship may not be reflected at the biomarker level. Nevertheless, the limited number of patients undergoing MEFV analysis warrants cautious interpretation of this finding.

Overall, our study suggests that the elevation of serum hBD-2 levels may be associated with IgAV independently of age, infection history, or acute phase reactants. In this context, hBD-2 may serve as a potential biomarker for IgAV. However, the lack of association between serum hBD-2 levels and gastrointestinal involvement raises the possibility that hBD-2 may provide more meaningful information in other biological samples, likely reflecting its localized tissue expression.

The limitations of our study include the fact that although the healthy control group was free of infection and steroid use, only steroid use could be excluded in the IgAV group. In addition, the time interval between symptom onset and sampling could not be standardized due to variability in hospital admission times. Furthermore, hBD-2 levels were not assessed following treatment or during remission, preventing evaluation of the biomarker's dynamic changes over the disease course.

Our study demonstrates that hBD-2 levels are elevated in IgAV and that this elevation may occur independently of classical inflammatory markers. These findings suggest that hBD-2 could serve as a complementary biomarker in IgAV; however, larger, prospective studies assessing hBD-2 in different biological samples are needed to clarify its diagnostic and clinical utility.

Conclusion

This study indicated that serum hBD-2 levels were significantly higher in children with IgA vasculitis compared to healthy controls. The fact that this elevation appeared independent of age, infection history, acute phase reactants, and MEFV mutations suggests that hBD-2 may reflect a disease specific immune response and could serve as a complementary biomarker in IgAV. The lack of association with gastrointestinal involvement raises the possibility that other biological samples may provide more meaningful insight. Further studies are required to better define the clinical utility of hBD-2.

Declarations

Ethics Committee/Consent To Participate: All subjects and their relatives gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Etlik City Hospital in 12/18/2024 (Number of Adjudication: AESH-BADEK-2024-1229).

Consent to Publication: This study doesn't include any kind of personal information about the patients, which was declared in ethics approval. Therefore, our research team didn't feel the need to receive consent for publication

Category Of Study: Basic Science

Impact: Serum Human Beta Defensin-2 levels elevate in children with IgA vasculitis independent of acute phase reactants. Human Beta Defensin-2 is known to be associated of urinary system infection and

inflammatory bowel diseases but no studies had been done before to investigate its role in IgA vasculitis. This study shows that there is a potential role of innate immunity in childhood vasculitis.

Large Language Model: We haven't used any kind of large language model

Declaration of Interests: The authors have no conflict of interest to declare.

Funding Support Statement :The authors declared that this study has received no financial support.

References

1. Oni, L. & Sampath, S. Childhood IgA Vasculitis (Henoch Schonlein Purpura)-Advances and Knowledge Gaps. *Front Pediatr* **7**, 257 (2019).
2. Xu, L., Li, Y. & Wu, X. IgA Vasculitis Update: Epidemiology, Pathogenesis, and Biomarkers. *Frontiers in Immunology* **Volume 13 - 2022** (2022).
3. Harder, J., Bartels, J., Christophers, E. & Schröder, J. M. A Peptide Antibiotic from Human Skin. *Nature* **387**, 861 (1997).
4. Schröder, J. M. & Harder, J. Human Beta-Defensin-2. *Int J Biochem Cell Biol* **31**, 645-651 (1999).
5. Singh, P. K. et al. Production of Beta-Defensins by Human Airway Epithelia. *Proc Natl Acad Sci U S A* **95**, 14961-14966 (1998).
6. Cobo, E. R. & Chadee, K. Antimicrobial Human B-Defensins in the Colon and Their Role in Infectious and Non-Infectious Diseases. *Pathogens* **2**, 177-192 (2013).
7. Tomita, T. et al. Molecular Mechanisms Underlying Human Beta-Defensin-2 Gene Expression in a Human Airway Cell Line (Lc2/Ad). *Respirology* **7**, 305-310 (2002).
8. Kota, S. et al. Role of Human Beta-Defensin-2 During Tumor Necrosis Factor-Alpha/Nf-KappaB-Mediated Innate Antiviral Response against Human Respiratory Syncytial Virus. *J Biol Chem* **283**, 22417-22429 (2008).
9. Shin, J. S., Kim, C. W., Kwon, Y. S. & Kim, J. C. Human Beta-Defensin 2 Is Induced by Interleukin-1beta in the Corneal Epithelial Cells. *Exp Mol Med* **36**, 204-210 (2004).
10. Chadebech, P. et al. Use of Human Reconstructed Epidermis to Analyze the Regulation of Beta-Defensin Hbd-1, Hbd-2, and Hbd-3 Expression in Response to Lps. *Cell Biol Toxicol* **19**, 313-324 (2003).
11. Mineshiba, J. et al. Transcriptional Regulation of Beta-Defensin-2 by Lipopolysaccharide in Cultured Human Cervical Carcinoma (Hela) Cells. *FEMS Immunol Med Microbiol* **45**, 37-44 (2005).
12. Bhat, S., Song, Y. H., Lawyer, C. & Milner, S. M. Modulation of the Complement System by Human Beta-Defensin 2. *J Burns Wounds* **5**, e10 (2007).
13. Niyonsaba, F., Ogawa, H. & Nagaoka, I. Human Beta-Defensin-2 Functions as a Chemotactic Agent for Tumour Necrosis Factor-Alpha-Treated Human Neutrophils. *Immunology* **111**, 273-281 (2004).

14. Yang, D. et al. Beta-Defensins: Linking Innate and Adaptive Immunity through Dendritic and T Cell Ccr6. *Science* **286**, 525-528 (1999).
15. Panda, S. et al. Protective Role of Human Beta-Defensin-2 and Cathelicidin in High Risk Close Household Contacts of Pulmonary Tuberculosis. *Clinical Immunology Communications* **3**, 23-30 (2023).
16. Shelley, J. R. et al. A Mechanistic Evaluation of Human Beta Defensin 2 Mediated Protection of Human Skin Barrier in Vitro. *Scientific Reports* **13**, 2271 (2023).
17. Jansen, P. A. et al. Beta-Defensin-2 Protein Is a Serum Biomarker for Disease Activity in Psoriasis and Reaches Biologically Relevant Concentrations in Lesional Skin. *PLoS One* **4**, e4725 (2009).
18. Dalcin, D. & Ulanova, M. The Role of Human Beta-Defensin-2 in Pseudomonas Aeruginosa Pulmonary Infection in Cystic Fibrosis Patients. *Infect Dis Ther* **2**, 159-166 (2013).
19. Zilbauer, M. et al. Expression of Human Beta-Defensins in Children with Chronic Inflammatory Bowel Disease. *PLoS One* **5**, e15389 (2010).
20. Koeninger, L. et al. Human B-Defensin 2 Mediated Immune Modulation as Treatment for Experimental Colitis. *Front Immunol* **11**, 93 (2020).
21. Roache-Robinson P, K. R., Hotwagner DT. (StatPearls [Internet], 2023).
22. Shawky, N. M., Youssef, T. M., Elshafie, A. I. M. & Ahmed, A. E. Role of Human Beta-Defensin 2 (Hbd2) in the Diagnosis and Monitoring Activity of Ulcerative Colitis. *QJM: An International Journal of Medicine* **117** (2024).
23. Gedik, A. H. et al. Cathelicidin (LI-37) and Human B2-Defensin Levels of Children with Post-Infectious Bronchiolitis Obliterans. *Clin Respir J* **11**, 243-247 (2017).
24. Routsias, J. G., Marinou, D., Mavrouli, M., Tsakris, A. & Pitiriga, V. Serum B-Defensin 2, a Novel Biomarker for the Diagnosis of Acute Infections. *Diagnostics (Basel)* **13** (2023).
25. Ece, A. et al. Neutrophil Activation, Protein Oxidation and Ceruloplasmin Levels in Children with Henoch-Schönlein Purpura. *Pediatr Nephrol* **22**, 1151-1157 (2007).
26. Ekinci, R. M. K. et al. Mefv Gene Variants in Children with Henoch-Schönlein Purpura and Association with Clinical Manifestations: A Single-Center Mediterranean Experience. *Postgrad Med* **131**, 68-72 (2019).
27. Cakici, E. K. et al. Mefv Gene Mutations in Children with Henoch-Schönlein Purpura and Their Correlations-Do Mutations Matter? *Clin Rheumatol* **38**, 1947-1952 (2019).

Table

Table 1: Human Beta Defensin 2 Levels According to Different Characteristics in Patients with Immunoglobulin A Vasculitis

	hBD-2 levels (pg/dL) (median)	p value
Male (n=18)	9536	0.903
Female (n=27)	9524	
Age >10 years (n=14)	13711	0.007
Age < 10 years (n=31)	8662	
High APR	9522	0.015
Normal APR	11317	
MEFV mutation present (n=10)	8667	0.371
Normal MEFV gene (n=10)	9662	
Mutations in exon 10 (n=7)	8662	0.260
Mutations outside exon 10 (n=3)	14167	
Abdominal involvement present	9525	0.436
No abdominal involvement	10410	
Steroid used	9525	0.862
Steroid spared	9967	
Pulse steroid used	9536	0.963
Pulse steroid spared	9525	

Figures

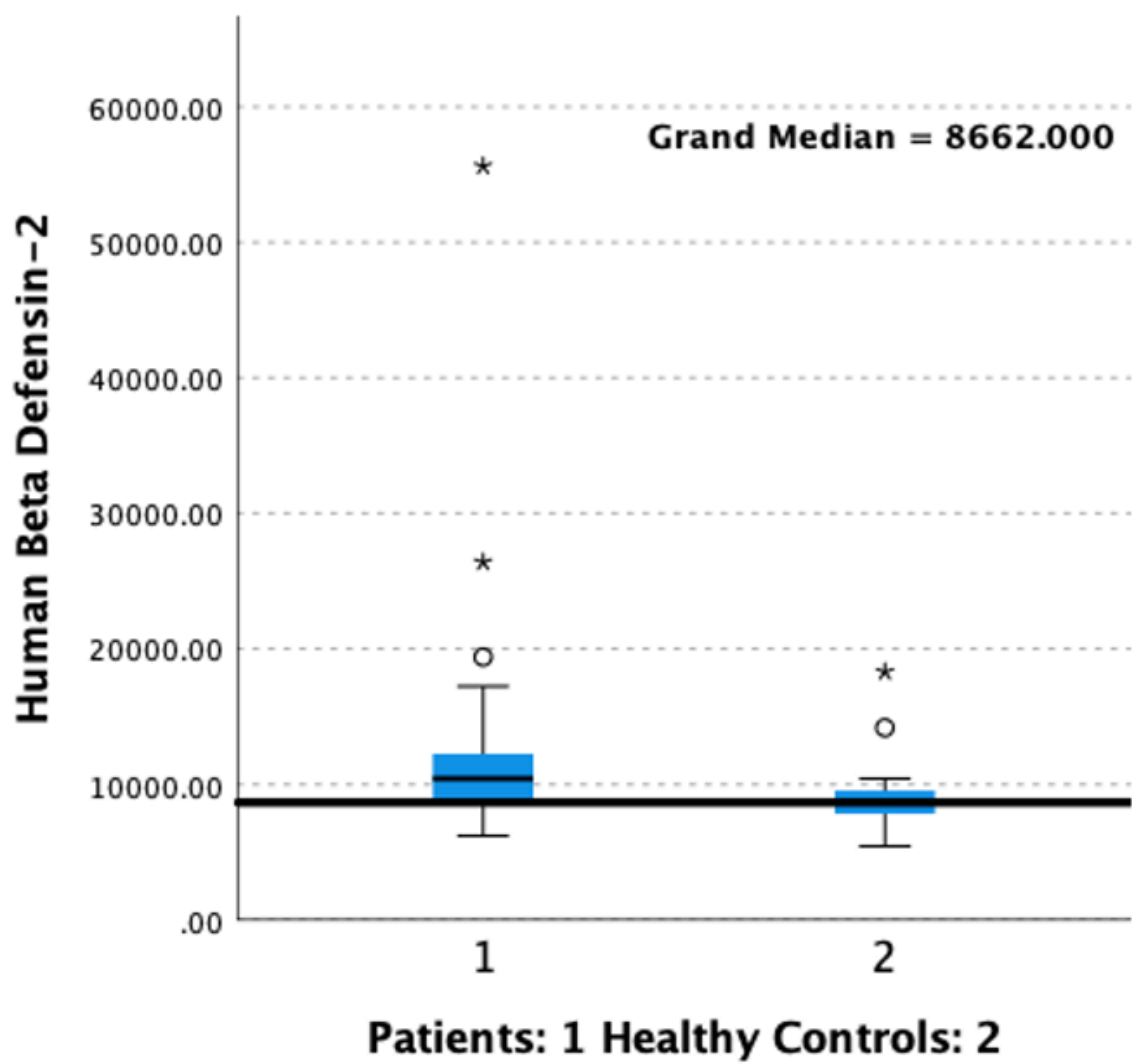


Figure 1

Human Beta Defensin-2 levels in Patients and Controls (median values)

Human Beta Defensin-2 Levels in patients with IgAV

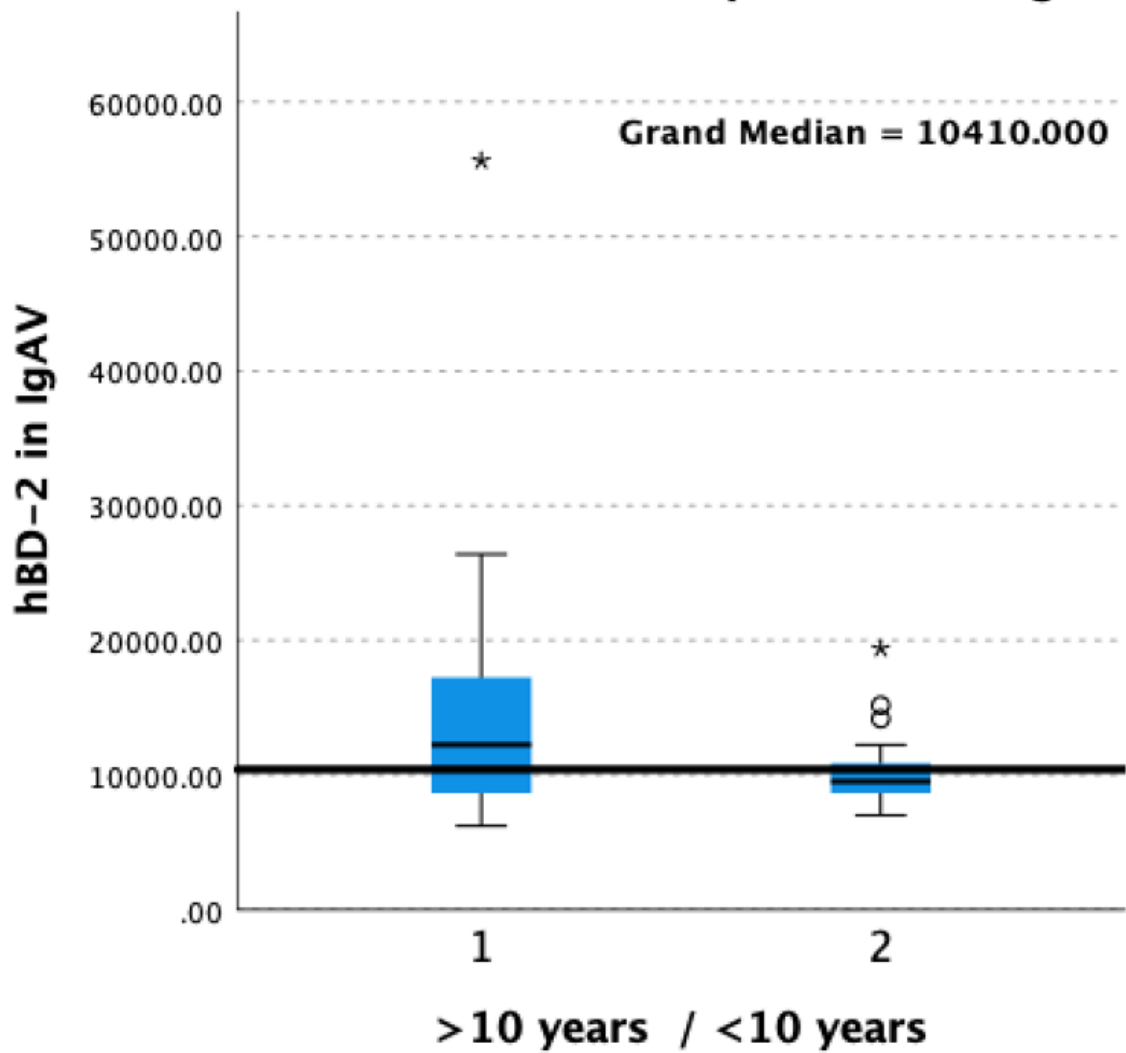


Figure 2

Difference of Human Beta Defensin-2 Levels in Patients with IgAV (median values) According to Age

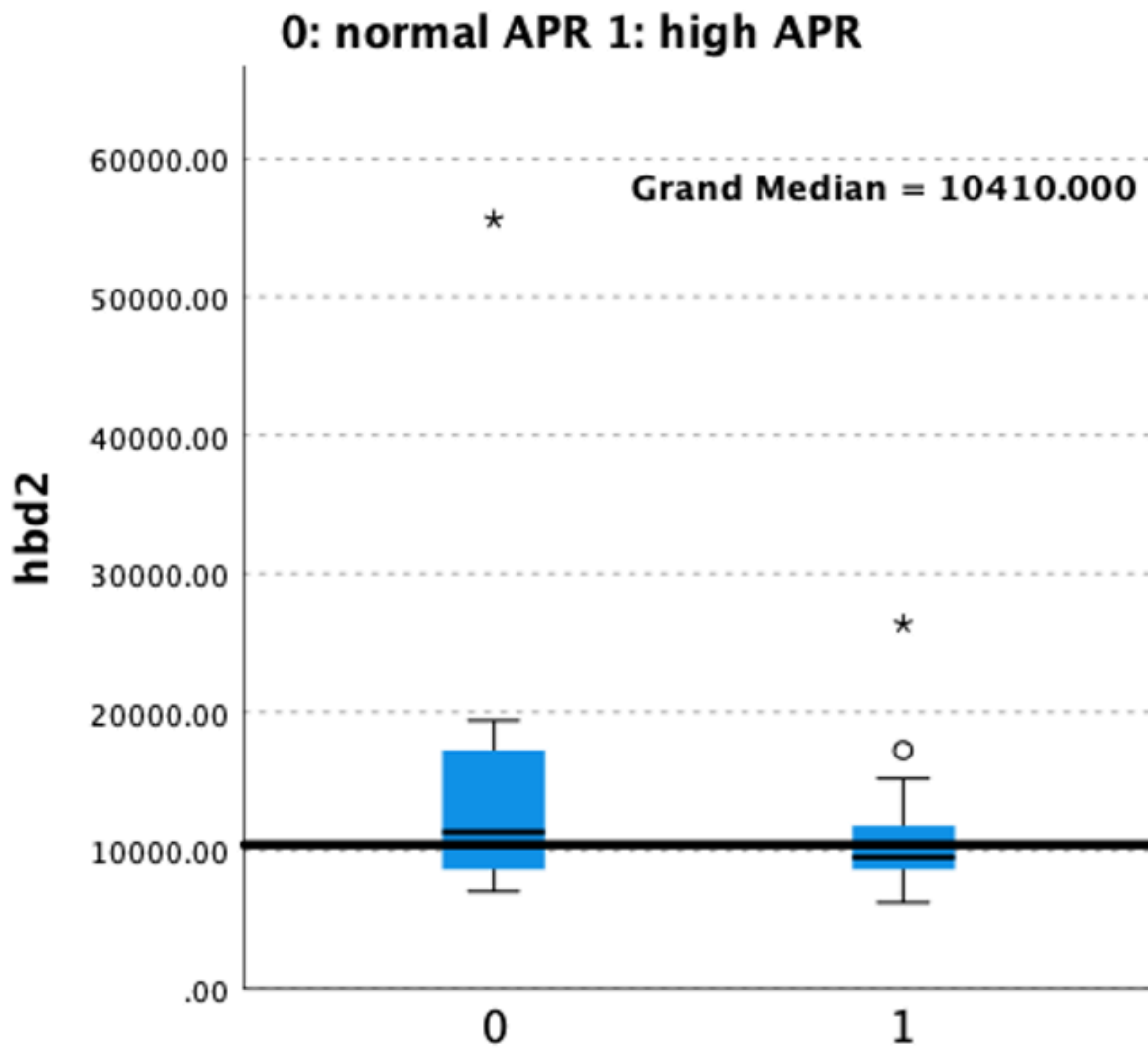


Figure 3

Association of Elevated Acute Phase Reactants and Human Beta Defensin-2 Levels in Patients with Immunglobulin A Vasculitis (median values)