Biochemistry and Biophysics Reports 46 (2026) 102621

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

Dysregulated serum levels of human tissue kallikreins, hK7 and hK10, as potential biomarkers of asthma

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ARTICLE INFO

Keywords:
Asthma
hK7
hK10
Biomarkers
Kallikrein protein
ELISA

ABSTRACT

Background: Asthma is a highly prevalent inflammatory disease worldwide with limited diagnostic and therapeutic approaches.
Objective: This study was conducted to explore the possible role of hK7 and hK10 in asthma progression and evaluate them as potential biomarkers of asthma.
Methods: In total, 74 patients with asthma and 99 normal controls participated in the study. Time-resolved immunofluorometric (TRIF) indirect back-titration (bt) ELISA was employed to measure the expression of hK7 and hK10 in both groups. Statistical analysis was performed using SPSS.
Result: Results indicated hK7 upregulation and hK10 downregulation in patients with asthma as compared to normal controls. Further non-significant association of hK7 and hK10 expression with patients'age was noted.
Conclusion: This study documents the true or impartial behavior of hK7 and hK10 being evaluated as biomarkers of asthma. The aberrant expression of these hKs can help to design diagnostic and therapeutic measures to control asthma. The associated factors need to be considered as they affect the expression of both KLK proteins. Further work is needed for the exploration and utilization of KLK proteins as drug targets fortherapy.

1. Introduction

Asthma is the most prevalent inflammatory disorder of the bronchial airways in humans [1]. Worldwide, about 300 million individuals are known to be affected by asthma, in addition to 100 million people predicted to be at risk. Moreover, asthma contributes to high mortality and morbidity rates, influencing the quality of life and resulting in premature death, an unavoidable health issue [2]. According to the World Health Organization (WHO), asthma affected nearly 262 million people in 2019 and led to a death rate of 455000 [3]. Pakistan, being a developing and sixth-most densely populated country, has a high likelihood of asthma and other allergic disorders. According to an approximation, about 20% Pakistan's pediatric population suffers from asthma. Here, poor health provision, more urbanization trends, lack of awareness about disease spread, consanguineous marriages, and increased exposure to pollutants contribute to enhancing the chance of asthma [4]. Both genetics and environmental factors, including air pollutants, fungal and viral infections, trigger and potentiate asthma [5].

As far as early diagnosis and prognosis are concerned, there is not a single generally accepted technique and ideal biomarker of asthma [6,

Abbreviations: (WHO), World Health Organization; (FeNO), Fractional exhaled nitric oxide; (KLKs), Kallikrein; (BAL), Bronchoalveolar lavage fluid; (KLK7), Kallikrein-related peptidase 7; (TRIF), Time-resolved immunofluorometric; (bt), Indirect back titration; (ELISA), Enzyme-linked Immunosorbent assay; ROC, (Receiver Operating Characteristic); (ρ), Spearman's rho; (τ), Kendall's tau.

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https://doi.org/10.1016/j.bbrep.2026.102621
Received 28 December 2025; Received in revised form 2 April 2026; Accepted 6 May 2026
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7]. At present, only a few tests/biomarkers, including serum IgE level, urinary leukotriene E_4, fractional exhaled nitric oxide (FeNO), cortisol suppression test, sputum eosinophils count, CBC/blood eosinophils, and high-resolution CT-scanning, are being used to discriminate patients with asthma from normal individuals, but their application in prognosis, diagnosis, and therapy is still disputable [7]. However, eosinophils and FeNO can be considered viable biomarkers for the detection of asthma remission in patients. Blood eosinophil levels can also be employed as a biomarker to determine inactive airway inflammation before planning treatment [8]. Earlier, more than 100 asthma-associated genes have been identified in various populations all over the world, but in Pakistan, hardly any studies have reported the genetic markers for the management and treatment of asthma [4]. Among the globally studied markers, Kallikrein (KLKs) are the ones that are well known for their role in airway response management. Tissue KLKs are a subgroup of serine proteases encoded by a multigene family comprised of 15 members of the serine proteases [9]. Except for KLK1, a tissue kallikrein, KLK2-KLK15 are kallikrein-related peptidases. They are usually present in the neutrophils, endocrine, and endothelial cells. The kinins produced by KLKs are the inflammatory mediators that contribute to smooth muscle contraction, arteriole dilations, and increased capillary membrane permeability [10] and have significant pro-inflammatory activities [11] and also found to be involved in different infectious, chronic, and tumor-associated lung disorders [12].

Various reports explain the pivotal pathophysiological function of Kallikreins (KLKs) in asthma [13]. KLK1, KLK3, and KLK14 have been implicated in asthma pathogenesis. KLK13 contributed to asthma progression by secreting interferon γ [14]. The hK7 was observed to be involved in the processing and activation of pro-interleukin 1 beta, which is known to be associated with asthma [15]. Both kinins and cytokines facilitate the pathophysiology of asthma [16]. In patients with mild atopic asthma, the increased KLK-like activity in the bronchoalveolar lavage fluid (BAL) further ensures the role of KLKs in asthma [17].

Although the role of various KLKs in asthma has been studied, to the best of our knowledge, at present, no work has been done to explore the function of Kallikrein-related peptidase 7 (KLK7), which is a serine protease having chymotrypsin-like activity [18], and KLK10, a secreted serine protease having trypsin-like activity [9]. Not a single study reported the hK7 and hK10 levels in asthma. Keeping in view the significance and contribution of various KLKs members in asthma and other inflammatory diseases, we initiated this study to screen differential expressions of hK7 and hK10, if any, in the sera of patients with asthma in comparison to normal individuals to evaluate the potential of hK7 and hK10 as biomarkers of asthma. We hypothesized that the serum hK7 and hK10 are dysregulated in Pakistani patients with asthma. The derived information will be fruitful in developing the KLK and kinins' inhibitors and their utilization in the therapy of tumors or inflammatory disorders like asthma.

2. Methods

2.1. Materials and chemicals

The monoclonal antibodies used during assay (hK7 (KLK7 (1407), sc-80148, Lot # 11610) and hK10 (KLK10 (1-06), sc-100551, Lot #C0708)) and substrate of alkaline phosphatase [i.e., diflunisal phosphate ($C_{13}H_9F_2O_6P$, Cat # sc-207575)] were synthesized by Santa Cruz Biotechnology Inc., USA. While purified hK7 peptides (Cat # ab41385, Lot # 275205) and hK10 peptides (Cat # ab41388, Lot # 275228) purchased from Abcam and goat anti-mouse IgG alkaline phosphatase conjugate manufactured by Calbiochem (Cat # DC05L) were employed. All other assay chemicals were of molecular biology grade and obtained from standard manufacturers like Merck/Sigma-Aldrich/Fluka/Biobasic.

2.2. Sample collection

A total of 74 blood samples were collected from patients with asthma at local hospitals and clinics, and 99 from healthy, normal residents of the study area, with informed written consent recorded on a demographic form following the guidelines of the Helsinki Convention (1992). The collected blood samples were transported on ice to the laboratory, and sera were extracted, aliquoted, and stored at −80 °C until further analysis.

2.3. Inclusion and exclusion criteria

Clinically diagnosed patients with non-atopic asthma and normal individuals with no asthma were enrolled in the study. The participants (patients with asthma and normal controls) who signed the consent form were included in the study. HBV/HCV-positive and HBV/HCV-negative patients with asthma were included. Whereas the participants who refused to provide written consent were excluded. Further, the participants suffering from any other disease were also excluded from the study. Smokers and patients with asthma using any medications like corticosteroids/oral steroids were not included.

2.4. Time-resolved immunofluorometric (TRIF) indirect back titration (bt) ELISA

The measurement of serum hK7 and hK10 levels in study groups (i.e., healthy individuals and patients with asthma) was accomplished through an in-house optimized, time-resolved immunofluorometric (TRIF) assay, which in principle was an indirect back-titration Enzyme-linked Immunosorbent assay (bt-ELISA) following our previously published protocol [19].

2.5. Preparation of calibration curve

For preparing calibration curve of purified hK7 and hK10 antigen, the hK7 and hK10 protein were diluted in dilution buffer (50 mM Tris-HCl, (pH 7.8) containing 20 g/L bovine serum albumin, 0.5 ml/L T ween 20) and calibrator of variable concentrations (i.e., 1, 2, 4, 5, 10, 20, 40, 50, 80, 100, 160, 320, 640, 1000, 1280, 2000 pg/100 μL) were obtained. Optimization of the calibration curve was done after six parallel runs of reproducible results, and each serum sample was run independently twice. The calibration curve for hK7 and hK10 is shown in Fig. 1.

2.6. Plate preparation

During the assay, the polystyrene microtiter plate wells were coated with capture antibody (anti-hK7/anti-hK10) in variable quantities (ranging from 100 to 500 ng). The serum samples were thawed and appropriately diluted (1:5 or 1:10) in dilution buffer (50 mM Tris-HCl, pH = 7.5) before analysis. The microtiter plate wells were coated with 100 μL of the capture antibody/well and incubated overnight at 4 °C. After incubation, the antibody solution was discarded, and the plates were washed with wash buffer (10 mM Tris-HCl buffer (pH 7.4) containing 150 mmol/L NaCl and 0.5 ml/L Tween 20). Next, 250 μL of blocking solution (50 mmol/L Tris-HCl buffer (pH 7.8), containing 30 g/L bovine serum albumin and 0.5 ml/L Tween 20) was added and incubated at 25 °C for 1 h. Plates were washed six times after removing the blocking solution and were ready for further use.

2.7. Quantification of hK7 protein expression

For the quantification of hK7 in serum samples, 100 μl of serially diluted serum samples (1:5 or 1:10 in dilution buffer) were added to the antibody-coated wells and incubated at 25 °C for 2 h with occasional shaking. After incubation, the plates were washed six times with the

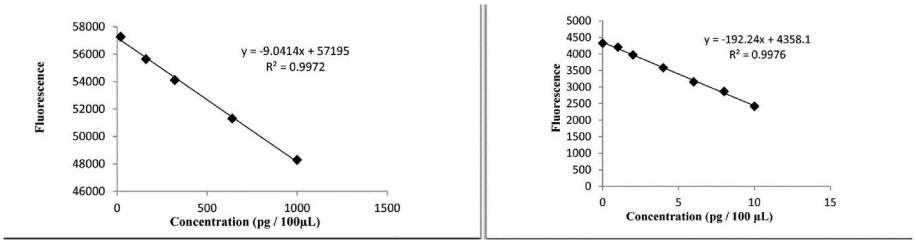

Fig. 1. Calibration curve for the quantification of hK by TRIF bt-ELISA. (A) Calibration curve for the quantification of hK7 (B) Calibration curve for the quantification of hK10.

wash buffer. Next, 100 µL of alkaline phosphatase-conjugated antibody (100 ng/1000 µL) diluted in the dilution buffer was added to each well following incubation at 25 °C for 2 h with occasional shaking. Washing was done six times. 100 µL of substrate solution (0.1 mol/L Tris-HCl, pH 9.1, containing 1 mmol/L diflunisal phosphate, 0.1 mol/L NaCl, and 1 mmol/L $MgCl_2$) was added and incubated for 10 min, then 100 µL of developing solution (1 mol/L Tris base, 0.4 mol/L NaOH, 2 mmol/L $TbCl_3$, 3 mmol/L EDTA) was added and fluorescence was measured within time of 2 min by ELISA plate reader Biotek Eslx800 in time-resolved mode with endpoint as the mode of measurement. Emission was taken at 590/35 while excitation was measured at 360/10, which gives the best fluorescence signal measurement using the top probe optics position. Total reading time was 100 µsec with a delay time of 20 µsec. A total of ten readings were taken per data point, with a delay of 1 msec between measurements, and 100msec for plate adjustment. Sensitivity adjustment setting was kept by default, and the sensitivity scale was adjusted according to well with highest concentration using Gen5 software.

The captured antibody concentration of 300 ng/well represented a detectable signal increment in a linear manner for hK7 and hK10 proteins and thus was opted for later protein quantification and screening in serum samples [19].

2.8. Quantification of hK10 protein expression

For hK10 quantification, microtiter plate wells were coated with 100 µl of serially diluted serum samples (1:5 or 1:10 in dilution buffer) following incubation at 25 °C for 2 h with occasional shaking. Washing was done six times, and 100 µL of the alkaline phosphatase-conjugated antibody (100 ng/1000 µL) diluted in the dilution buffer was added to each well. Plates were incubated at 25 °C for 2 h with shaking after a short time interval. Wells were washed six times, and 100 µL of the substrate solution (0.1 mol/L Tris-HCl, pH 9.1, containing 1 mmol/L diflunisal phosphate, 0.1 mol/L NaCl, and 1 mmol/L $MgCl_2$) was added and incubated for 10 min. After incubation, 100 µL of developing solution (1 mol/L Tris base, 0.4 mol/L NaOH, 2 mmol/L $TbCl_3$, 3 mmol/L EDTA) was added and, reading was taken within 2 min following the procedure described earlier for hK7 quantification.

2.9. Detection limit, precision, and linearity of the assay

The precision of the assay was evaluated for hK7 and hK10 standards within runs and in between runs. The percentage coefficient of variation (CV) was found to be consistent with the recommended CV range, i.e., 2 % - 9 % of immunoassay. The linearity of the assay was measured using 2-3 dilutions of clinical samples, i.e., 1: 10, 1:5, and, if required, 1:2.5 dilutions as well. The lowest measured concentration was 2 pg/100 µl (20 ng/l), and thus, a concentration below this cannot be distinguished from the blank.

2.10. Statistical analysis of data

For the statistical analysis, SPSS 18.0 and MedCalc 12.2.0.0 were used. First, the normal distribution of the data was evaluated through the D'Agostino-Pearson test. The independent sample *t*-test (for normally distributed/Gaussian data) or Mann-Whitney *U* test (for non-Gaussian data) was used to calculate the p-value.

The ROC (Receiver Operating Characteristics) curve and logistic regression analysis (both univariate and multivariate) helped to deduce the diagnostic potential of hK7/hK10 measurement for predicting asthma. The correlation and logistic regression analysis were carried out. For non-Gaussian data, Spearman's rho (ρ) and Kendall's tau (τ) rank correlation coefficients were computed. While for Gaussian or normally distributed data, the Pearson correlation coefficient was calculated using our previously published method [20]. The methodology adopted has been summarized in a graphical abstract.

3. Results

3.1. Quantification of hK7 and hK10 in the sera

The analysis of 74 patients sera, i.e., female (n = 33) and male (n = 41), by TRIF bt-ELISA shows that hK7/hK10 level in serum was below the detectable limit in a few patients and normal samples (Supplementary Table 1). The D'Agostino-Pearson test indicated rejection of normality for hK7 ($p < 0.0001$) and hK10 ($p = 0.0181$). The Mann-Whitney *U* test indicated significant ($p < 0.0001$) upregulation of hK7 and significant ($p < 0.0001$) downregulation of hK10 in the total patients with asthma as compared to the normal controls (Fig. 2 and Supplementary Table 2).

3.2. Expression analysis of hK7 and hK10 based on clinicopathological features

The expression analysis was performed based on various factors, including gender, age, family history of the disease, time duration of disease, and HBV/HCV infection.

3.3. Expression level of hK7 and hK10 based on gender

The results of the D'Agostino-Pearson test declared hK7 quantification data as non-Gaussian, while hK10 quantification data as Gaussian. All comparisons were carried out among gender-specific groups. In all female and male patients, hK7 level was significantly upregulated while the hK10 level was significantly downregulated (Figs. 3 and 4, Supplementary Table 3).

The ROC curve analysis highlighted increased hK7 and decreased hK10 serum levels as a diagnostic tool that can be potentially applied to discriminate male and female patients with asthma from healthy male and female subjects, respectively ($p < 0.0001$; Figs. 5 and 6).

Similarly, the univariate and bivariate logistic regression analysis validated the upregulation of serum hK7 ($p < 0.0001$) and downregulation of hK10 ($p < 0.001$) as better independent asthma markers for the prediction of asthma in both male and female patients (Table 1).

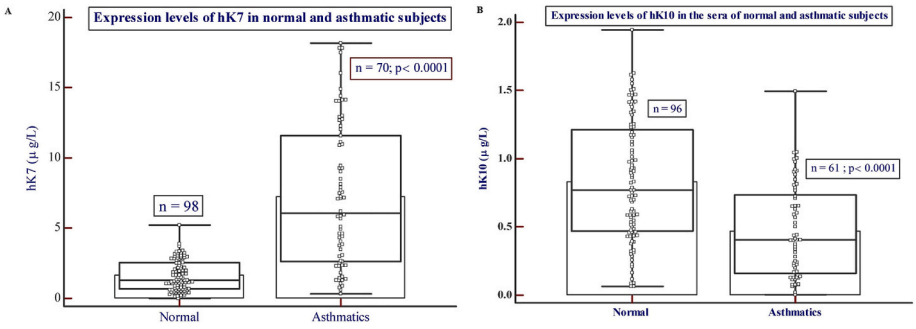

Fig. 2. A comparison of hK7 and hK10 concentrations in the serum of total normal and patients with asthma (A) hK7 concentration (B) hK10 concentration. In the graph, the horizontal line extends from the minimum to the maximum, the central box represents the values from lower to the upper quartile (i.e., 25 to 75 percentile), middle line of the central box represents the median, outside box indicates error bars for the mean, small box along the horizontal line directly corresponds to the distribution of samples w.r.t serum hK7 and hK10 concentration.

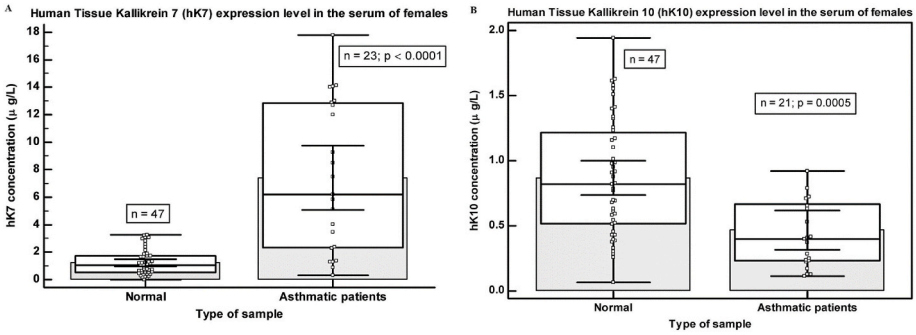

Fig. 3. A comparison of hK7 and hK10 concentrations in the serum of normal and female patients with asthma (A) hK7 concentration in females (B) hK10 concentration in females. In the graph, the horizontal line extends from the minimum to the maximum, the central box represents the values from the lower to upper quartile (i.e., 25 to 75 percentiles), the middle line of the central box represents the median, and the horizontal line inside the central box represents 95% CI for the mean. The dotted area indicates error bars for the mean; the small box along the horizontal line directly corresponds to the distribution of samples with respect to serum hK7 and hK10 concentrations.

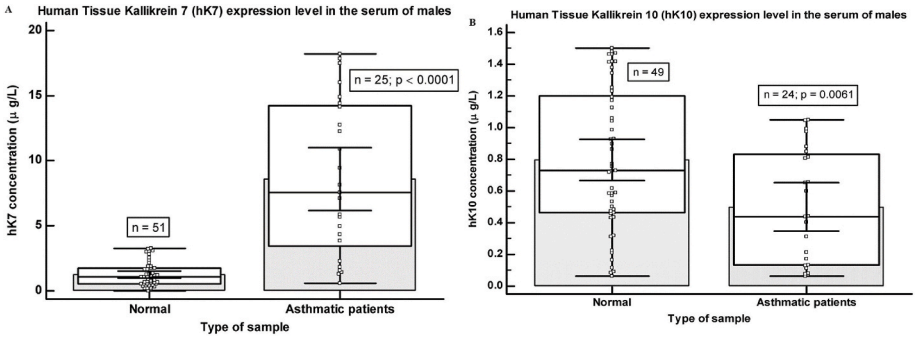

Fig. 4. A comparison of hK7 and hK10 concentration in the serum of normal and male patients with asthma (A) hK7 concentration in males. (B) hK10 concentration in males**.** In the graph, the horizontal line extends from the minimum to the maximum, the central box represents the values from the lower to the upper quartile (i.e., 25 to 75 percentiles), the middle line of the central box represents the median, and the horizontal line inside the central box represents 95% CI for the mean. The dotted area indicates error bars for the mean; the small box along the horizontal line directly corresponds to the distribution of samples with respect to serum hK7 and hK10 concentrations.

3.4. Expression level of hK7 and hK10 based on age

The patient group included subjects aged 11- 70 years. To investigate the impact of age on hK7 and hK10 expression levels, patients were subdivided into different age groups, each of 10 years. The serum hK7 expression level was significantly up-regulated ($p < 0.05$) in all age groups, i.e., up to 40 years of age in female and 30 years of male patients ($p < 0.05$). While hK10's expression levels were significantly low in younger patients with asthma, particularly in 21-30 years old female patients ($p = 0.0119$) and 11-20 years old male patients group ($p = 0.0102$). Data has been summarized in Supplementary Table 4 and Supplementary Table 5.

A weak negative correlation between age and hK7 ($p < 0.05$) serum levels, among normal and patients, was highlighted by the coefficients of determination (R^2), Spearman's rho (ρ), and Kendall's tau (τ) correlation coefficients. A statistically non-significant ($p < 0.001$) negative correlation between age and hK10 expression levels in both male and female patients has been reflected by Pearson's correlation coefficient.

Bivariate analysis revealed hK7 and hK10 expression levels as better independent markers of asthma in relation to the age of the patients

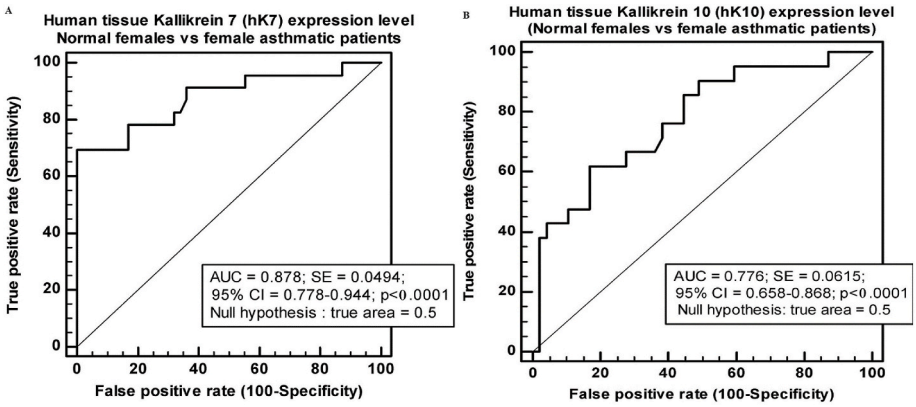

Fig. 5. ROC curve analysis of hK7 and hK10 quantification for discrimination between normal and female patients with asthma (A) ROC curve analysis of hK7 in females (B) ROC curve analysis of hK10 in females. AUC was analyzed by Hanley and McNeil, 1982 method, CI is a binomial exact confidence interval.

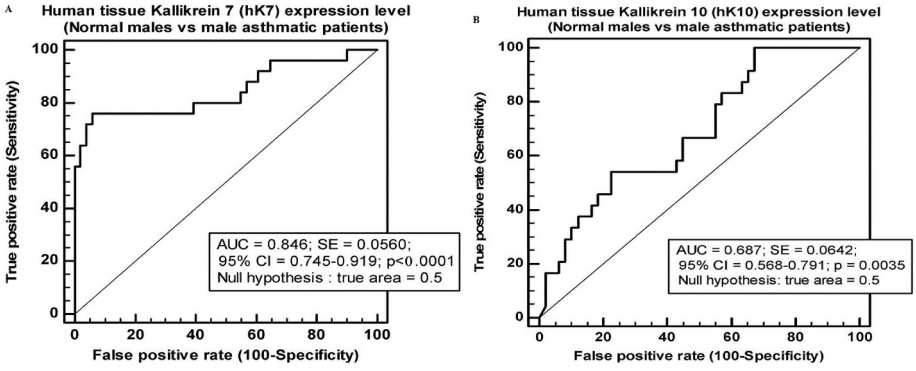

Fig. 6. ROC analysis of hK7 and hK10 quantification for discrimination between normal males and patients males (A) ROC curve analysis of hK7 in males (B) ROC curve analysis of hK10 in males. AUC was analyzed using the Hanley and McNeil, 1982 method; CI is a binomial exact confidence interval.

Table 1
Logistic regression analysis of hK7 and hK10 expression level for predicting the presence of asthma in patients.

Samples	Proteins	Variables	Crude odd ratio	SE	95% CI	p-value	Coefficient	Overall Model Fit
Females	**hK7**	**Univariate Analysis**						
		Log [hK7]	65.684	1.011	9.058-476.302	<0.0001	4.185	$p < 0.0001$
		Age	1.0057	0.016	0.9738-1.0385	0.7307	0.006	$p = 0.7314$
		Bivariate Analysis						$p < 0.0001$
		Log [hK7]	88.633	1.095	10.356-758.445	<0.0001	4.484	——
		Age	1.0304	0.024	0.983-1.0797	0.2097	0.03	——
	hK10	**Univariate Analysis**						
		Log [hK10]	0.0617	0.909	0.0104-0.3665	0.0022	−2.785	$p = 0.0002$
		Age	1.01	0.017	0.9771-1.0441	0.5551	0.01	$p = 0.5570$
		Bivariate Analysis						$p = 0.0009$
		Log [hK10]	0.0619	0.091	0.0104-0.3679	0.0022	−2.783	——
		Age	1.0095	0.0184	0.9737-1.0466	0.6075	0.0095	——
Males	**hK7**	**Univariate Analysis**						
		Log [hK7]	87.901	1.064	10.929-706.975	<0.0001	4.476	$p < 0.0001$
		Age	0.982	0.023	0.938-1.0280	0.4413	−0.018	$p = 0.4332$
		Bivariate Analysis						$p < 0.0001$
		Log [hK7]	87.567	1.07	10.749-713.389	<0.0001	4.472	——
		Age	0.987	0.029	0.931-1.0452	0.6492	−0.013	——
	hK10	**Univariate Analysis**						
		Log [hK10]	0.1779	0.664	0.0484-0.6537	0.0093	−1.727	$p = 0.0051$
		Age	0.9887	0.023	0.9452-1.0343	0.6217	−0.011	$p = 0.6186$
		Bivariate Analysis						$p = 0.0195$
		Log [hK10]	0.1799	0.667	0.0487-0.6648	0.0101	−1.715	——
		Age	0.9955	0.024	0.9496-1.0437	0.8522	−0.004	——

Data was evaluated at CI = 0.95.

(Table 1).

3.5. Expression level of hK7 and hK10 based on family history

The hK7 serum level was significantly higher, and the hK10 level was

significantly lowered in all patients with asthma, regardless of the status of family history. (Supplementary Table 6).

3.6. Expression level of hK7 and hK10 based on the duration of disease

Results showed a significant upregulation ($p < 0.05$) of hK7 and a downregulation of hK10 in female patients, irrespective of disease duration. While variation in hK7 expression levels was highly significant in male patients suffering from asthma either since birth ($p = 0.0006$) or for the last 1-10 years. Similarly, hK10 expression was significantly lower ($p = 0.0016$) in male patients with asthma for the last 1-5 years but was found to vary non-significantly ($p > 0.05$) in males suffering from asthma for either less than 1 year or more than 5 years (Supplementary Table 7).

3.7. Expression level of hK7 and hK10 based on HBV/HCV infection

The hK7 serum level was significantly elevated ($p < 0.005$) in HBV/HCV-positive male and female patients when compared with gender-specific normal and HBV/HCV-positive controls. However, the hK10 expression levels were significantly lower in HBV/HCV-positive male patients ($p < 0.005$) while the variation was non-significant ($p > 0.05$) in the case of HBV/HCV-positive gender-specific controls (Supplementary Table 8).

4. Discussion

It's our worst luck that asthma is still a highly prevalent and untreatable disease worldwide, including Pakistan, affecting millions of individuals all over the world [21]. The prevalence rate is increasing at an alarming rate, and it is estimated that by 2025, the number of patients with asthma will rise to 400 million. Keeping in view the alarming situation, the present study was designed to investigate new biomarkers, i.e., hK7 and hK10, of asthma. Results of our analysis depicted upregulation of hK7 and downregulation of hK10 in patients with asthma in relation to normal controls. Previously, no study has explored the expression of these KLK proteins in patients with asthma. However, different studies have shown the involvement of KLK7 and KLK10 in other diseases. As in adenocarcinoma, downregulation of KLK7 expression ($P = 0.003$) was reported [22]. Earlier, KLK7, along with KLK5, was observed to be linked with inflammatory skin disease development with barrier defects, e.g., atopic dermatitis (AD) and Netherton syndrome [9]. It was noted that in skin inflammation, upregulation of KLK7 with KLK5 involves cleaving LL-37 into inflammatory peptide fragments, called the primary pathogenetic factor in rosacea [9]. Previously, KLK7 antimicrobial activity has been studied [9], and it was noted that KLK7 uses several mechanisms, like cathelicidin antimicrobial peptide precursors and pro-IL-1β, to regulate inflammatory pathways. KLK7 is recognized as a unique target of therapy in NS and AD, and KLK7 and KLK5 inhibitors serve as a drug [23].

Even though no direct involvement of KLK7 with asthma has been explored before, there are several documents explaining the role of KLK7 in inflammation development via different processes that could serve to unveil the significance of KLK7's aberrant expression as an asthma biomarker. Asthma is a heterogeneous disorder with a broad range of phenotypes. The two most common types are allergic asthma resulting from T-helper 2 (Th2 dominant) and non-atopic asthma caused by immune cells, i.e., neutrophils [24]. In AD lesions, it was observed that inflammatory cytokines induce KLK7 expression in keratinocytes. Among Th2 cytokines, IL-4 was known to greatly enhance the KLK7's activity [25]. Th2 cytokines are usually expressed by different immune cells, such as mast cells, eosinophils, Th2 lymphocytes, and basophils involved in the differentiation of Th2 cells, recruitment of eosinophils, and the production of IgE [25]. All these immune cells are involved in asthma, in which more than sufficient eosinophils, T-helper lymphocytes, and mast cells are produced that secrete inflammatory mediators (chemokines, cytokines, immunoglobulins, growth factors, histamine, and lipid mediators), which further trigger remodeling, mucous secretion, and bronchoconstriction [26].

Th2 cytokines are also involved in asthma inflammation and production of the inflammatory mediators, i.e., cytokines and chemokines [26]. In our study, we have documented the upregulation of KLK7 in patients with asthma, which might be in response to high Th2 levels. It represents a link between KLK7 and asthma and supports our result of KLK7 as an asthma biomarker.

Plasma and tissue kallikreins contributed to the formation of Bradykinin and kallidin, respectively. These peptides of kinins, i.e., bradykinin and kallidin, have been reported as mediators of inflammation and also trigger the release of other mediators [27]. Both these peptides required different peptidases for activation. Kallidin cleavage by aminopeptidase generates bradykinin [28]. Pieces of evidence are there proving the role of bradykinin in asthma, particularly by playing a part in airway sensory nerves activation and sensitization [29]. Bradykinins are recognized as major pro-inflammatory molecules activated in response to viral and allergic stimuli. They are known to affect the upper and lower airways, where their receptors B1 and B2 are present on immune cells. Other inflammatory mediators that regulate bradykinin expression using NF- Kb and MAPK signaling mechanisms include IL-4, IL-8, IL-13, and TNF-α, which are also associated with the recruitment of neutrophils and eosinophils. Bradykinins participate in chronic airway inflammation and remodeling (indicating severe asthma) by activating both epithelial and endothelial immune cells and mesenchymal cells [30]. Hence, these are the major bronchoconstrictors that have a strong association with asthma [31].

Likewise, on KLK10, no work has been done to reveal its diagnostic or therapeutic role in asthma, and no study has reported the expression analysis of KLK10 in asthma. However, KLK10 was studied as a potential therapeutic biomarker of lung disorders. KLK10 is known to be a tumor suppressor as it reduces the proliferation and invasion of malignant cells [12]. Various studies have described the upregulation and downregulation of KLK10 expression in different cancers. Its upregulation was noted in gastrointestinal tumors, oral squamous cell carcinoma, colorectal cancer, ovarian cancer, uterine papillary serous carcinoma, and pancreatic ductal adenocarcinoma, representing its role as an oncogene, while its downregulation was observed in prostate cancer, lung cancer, renal carcinoma, breast cancer, and non-small cell lung cancer [32]. Many mechanisms regulate the KLK10 expression. In cancer, KLK10 activation is hormone-dependent, and upregulation is mediated by various steroid hormones, i.e., androgens and estrogens, which are regulated by PI3K/Akt and Ras/MEK/ERK signaling pathways [33]. It was also speculated that steroid hormones regulate KLK expression. Among them, KLK 5, 6, 8, 10, and 11 were found to be regulated by Glucocorticoid [34]. Glucocorticoids are anti-inflammatory agents that aid in inflammation suppression by various mechanisms [35]. Glucocorticosteroids are widely used as an anti-inflammatory drug in asthma [24]. Our findings revealed downregulation of hK10 along with hK7 upregulation in patients with asthma. These agree with an earlier study where KLK10 is documented as an anti-inflammatory protein, and its downregulation has been reported in another inflammatory disease named Atherosclerosis [36].

It is known that different factors play a role in triggering asthma, including family history, less physical activity, pollution, infections, genetic makeup, smoking, and stress [21]. Therefore, we have also analyzed KLK expression based on different factors. First, both the studied KLK protein expressions were evaluated on a gender basis, and upregulation of hK7 was observed in females, while hK10 downregulation was noticed. Statistical analysis results help to document hK7 and hK10 as diagnostic biomarkers of asthma, which will aid in distinguishing patients with asthma and non-asthma. Formerly, no correlation of gender with KLK7 and KLK10 was studied, but the male gender was found to be an independent factor of asthma [37].

Association of KLK protein expression with age indicates hK7

upregulation in the 11-40 years age group females and 11-30 years age group male patients. Whereas hK10 downregulation was in 21-30 years age female patients. Further, a non-significant correlation between hK7 and hK10 expressions suggests that increasing age does not relate to the downregulation of hK expression. This proposed hK7 and hK10 to be age-independent biomarkers of asthma. Even though no association of hK7 and hK10 with age was found to be studied in asthma, it was observed that age was independently associated with asthma worsening [37]. Asthma family history was also considered a risk factor for asthma, which supports our investigation of significant upregulation of hK7 in patients with an asthma family history. But hK10 down-regulated expression was significant in males with an asthma history and females with no asthma history. In a study on cancer, KLK7 expression was stated to be associated with the age of patients, i.e., higher expression in the 41-60 year age group and stages of cancer [38] which exhibited that age should be considered as a factor of KLK's expression abnormality.

Duration of disease was also studied to evaluate the prognostic significance of KLK7 and KLK10. hK7 upregulation and hK10 downregulation were examined to be significant and independent of disease duration, which suggests that hK7 and hK10 are poor prognostic biomarkers of asthma. Lastly, we have also investigated the effect of hepatitis viral infection on hK7 and hK10 expression, which documented that HBV/HCV infection is involved in the upregulation of hK7 and downregulation of hK10 expression, as HBV/HCV-infected patients with asthma represent the same relevance to HBV/HCV normal control. HCV infection (chronic) has been reported to be an independent factor that leads to the worsening of asthma [37] and a significant association (OR: 1.28, 95% CI: 1.05 to 1.58) of HBV with asthma was also reported [39]. But no work was done earlier to explore the HCV and HBV association with aberrant KLK7 and KLK10 protein expression. In patients with asthma infected with HCV, high neutrophil percentages were observed, which further supports its role in more extreme airway inflammation [40]. Although no report demonstrates HBV and HCV association with KLK 7 and KLK10, there is evidence describing KLK10 promoter methylation correlation with HCV and HBV infection [41]. These findings suggest that hK7 upregulation and hK10 downregulation can be employed as asthma biomarkers.

5. Conclusion

In conclusion, our study reports the dysregulated expression of hK7 and hK10 proteins in Pakistani patients with asthma, and thus suggests that altered expression levels of hK7 and hK10 can be employed as diagnostic biomarkers for asthma. Further, there is a need to explore the therapeutic potential of hK7 and hK10.

Human ethics and consent to participate

The present study was approved by the Advanced Studies and Research Board (ASRB) and the Ethics and Research Committee of the Islamia University of Bahawalpur via letter no 55/ASR&B. All human experimental research was conducted in accordance with the Helsinki Declaration. Informed consent was obtained from all the participants.

Funding source

Higher Education Commission, Pakistan, awarded indigenous scholarship / fellowship to Dr Samina Ejaz (Grant/Award Number: 161017-Ps3-122).

CRediT authorship contribution statement

Samina Ejaz: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing – original draft. **Faiz-ul-Hassan Nasim:** Conceptualization, Project administration, Supervision, Validation, Writing – review & editing. **Iqra Abdullah:** Writing – review & editing. **Sami Ahmad:** Validation, Visualization. **Muhammad Ashraf:** Data curation, Supervision, Validation.

Declaration of competing interest

The authors declare that they have no conflict of interest associated with the research presented in this manuscript.

Acknowledgement

We are thankful to the Higher Education Commission (HEC), Pakistan for providing funds for this research.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2026.102621.

Data availability

The data supporting the findings of this study are available in the supplementary material of this article.

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