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Research Article

Association of rs242940 and rs242941 SNPs of *CRHR1* gene and plasma levels of IFN-γ, IL-6, IL-10 and IL-22 cytokines in asthma

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Abstract
Background: *Polymorphism in corticotrophin releasing hormone receptor 1 (CRHR1) gene, a key regulatory component of hypothalamic-pituitary-adrenal (HPA) axis and its correlation with the levels of various T-helper (Th) cell cytokines which have the major role in inflammatory response in asthma was not studied earlier. We hypothesised that polymorphism in CRHR1 gene may be associated with the concentration of various Th cytokines.*
Objective: *The aim of study was to determine the polymorphism in CRHR1 gene and their correlation with the expression of IFN- γ, IL-6, IL-10 and IL-22 cytokines in asthma.*
Methods: *Sequencing of CRHR1 gene single strand gene amplicons of specifically designed primers to determine the polymorphism and ELISA to evaluate the cytokine levels in 35 North Indian study subjects in each asthma patient (AP) and healthy control (HC) groups were carried out. Plasma separation and DNA isolation from whole blood packed cells were performed.*
Results: *Two single nucleotide polymorphism (SNP) (rs242941 and rs242940) were determined in sequenced region of CRHR1 gene in North Indian population. The plasma levels of IFN- γ and IL-6 were to be significantly elevated and levels of IL-10 were significantly decreased and IL-22 were non significantly increased in AP as compared to HC group. Correlation between forced expiratory volume in one-second (FEV1) % (predicted) and cytokines concentrations were also performed to analyse the severity of disease.*
Conclusion: *None of the allele of SNP was found significantly associated with asthma but genotypes of SNPs of CRHR1 gene weresignificantly correlated with FEV1% (predicted) and concentration of IFN- γ and IL-6 cytokines.*
Keywords: *Asthma, Polymorphism, CRHR1, Cytokine, ELISA and FEV1%, SNP.*

Introduction

Asthma is a chronic inflammatory disorder of the airways associated with increased airway hyper-responsiveness, recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or early morning[1]. According to the Global Asthma Report (2018), asthma affects approximately 339 million people worldwide[2]. Asthma creates a substantial burden, as it is more often under-diagnosed and under-treated[3]. It was seen as one of the leading causes of morbidity and mortality worldwide as well in India[4,5]. In India, there is a considerable variation in the prevalence of asthma, and the overall incidence was reported by 4.19%[5]. Asthma is a multifactorial genetic disease[6] involving gene-gene interactions and gene-environment interactions including infection (viral, bacterial), allergen exposure, pollution and smoking[7-8]. Corticotrophin releasing hormone receptor 1 (CRHR1) is the key regulatory components of the Hypothalamic-Pituitary-Adrenal (HPA) axis and response to stressful events, mediating the action of corticotrophin-releasing hormone (CRH) which is released from the hypothalamus[9].

Therefore, *CRHR1* was chosen as a candidate gene because the translational product of this gene is the key regulator of the HPA axis in pathophysiology of inflammation in asthma and the production of endogenous cortisol in response to stress[10,11]. The HPA axis and the sympathetic nervous system are the peripheral limbs of the stress system, the function of which is to maintain basal and stress-related homeostasis. The stress system responds to many distinct signals, including humoral and neural signals from the immune and inflammatory reaction[12]. Stress system–immune reaction interactions are undoubtedly complex and take place at multiple levels, whereas various immune-mediators have a crucial role in the initiation and propagation of immune responses. Th cell cytokines imbalance plays a pivotal role in the development and pathophysiology of asthma[13]. In this study, we aimed to investigate the association of SNP rs242940 and rs242941 in the *CRHR1* gene and their correlation with the plasma levels of Interferon (IFN)-ϒ, IL-6, IL-10 and IL-22 cytokines in asthma in North Indian population.

Materials and Methods

Study Subjects

In this case-control study, two study groups healthy control (HC) and asthmatic patient (AP) were included. Thirty-five North Indian subjects were enrolled in each study group. The patients were enrolled from the outpatient department of the Viswanathan Chest Hospital, Vallabhbhai Patel Chest Institute, Delhi. The diagnosis of the disease was categorised on the basis of family history, clinical symptoms, the onset of illness, some diagnostic tests like Pulmonary Functioning Test (PFT), etc. After diagnosis, asthmatic patients with $60 \leq$ FEV1% (predicted) and change in FEV1% (predicted) ≥ 12 except severe cases (according to EPR3 guidelines)[14] in age range between 20-60 years of either sex with no history of any systemic disease, e.g., hypertension, diabetes etc. and nonsmokers were enrolled for study. Pregnant and lactating females were excluded. Another group of healthy control subject was recruited from the same socio-economic-geographical background matched with asthma patient group of subjects for age and sex with no health problems and no history of asthma. Their lifestyles were also similar to those of the asthmatic individuals. The approval from the Institutional Ethics Committee of Vallabhbhai Patel Chest Institute, Delhi was obtained prior to the start of the study.

Collection of Blood Samples

Five ml of peripheral blood sample was collected from each subject in a screw-capped tube (Tarsons Products Pvt. Ltd., Kolkata, India) that contained 40µl of 0.5*M* EDTA. The specimen was transported to the laboratory on ice. Before drawing blood, informed written consent was obtained by each participant.

Separation of plasma and Isolation of genomic DNA

The blood sample was centrifuged (Centrifuge 5810R, Eppendorf, Hamburg, Germany) at 1200*g* for 10 minutes at 4ºC and supernatant of plasma was separated carefully to avoid mixing of any cells or other blood components using the pipette. Aliquots of plasma samples were stored at -80ºC (Ultra-Low Temperature Freezer, eppendorf) for further ELISA of cytokine study. After separation of plasma, genomic DNA was isolated from packed whole blood cells using the salting-out method by Miller *et al.*[15]. DNA pellet was dissolved in Tris-EDTA (TE) buffer followed by quantification of DNA at 260 nm in Nanovue spectrophotometer (Model: NanoVue Plus, GE Health Care Biosciences, Little Chalfont, UK). Finally, isolated DNA sample was electrophoresed on 0.8% agarose gel containing ethidium bromide (EtBr) and visualised under a Gel Documentation System (Bio-Rad Molecular Imager XR+, California, USA).

Primer designing

he primer sequence was determined by the programmer Primer 3 tool available on NCBI. Primer (forward; 5-GTGCTGTTTCCTGAGAGACTG-3, reverse; 5-CCCTTCTTTCTCTGCCTTTTAG-3, amplicon size, 267bp) for sense and antisense strands of *CRHR1* gene was designed that specifically recognise the specific sequences.

Determination of *CRHR1* gene polymorphism

To determine the polymorphism genomic DNA was amplified by polymerase chain reaction (PCR) initially using a primer pair. The PCR mixture contained 50 ng genomic DNA, 0.5 µ*M* of each primer (Sigma Aldrich, USA), 10µ*M* each dNTP, 1X PCR buffer and 1.0 unit Taq DNA polymerase. The amplification cycle was performed on a gradient thermal cycler (model: FTGRAD5D, Techne Cambridge Ltd). After initial denaturation at 94°C for 5 minutes, the DNA was amplified by 36 cycles: denaturation at 94°C for 45 seconds, annealing at 63.2°C for 45 seconds, and elongation at 72℃ for 45 seconds followed by a final elongation at 72℃ for 10 minutes. Amplified PCR products were separated by agarose gel electrophoresis on a 1.5% agarose gel and visualized under ultraviolet light after EtBr staining in Gel Documentation system. The amplicons were then cleaned up by using 10 units of Exonuclease-I and 10 units of thermo sensitive Alkaline Phosphatase at 37℃ for 30 minutes and 80℃ for 15 minutes for their further use in sequencing PCR as template DNA. Sequencing PCR was performed using ready to use BigDye™ Terminator v3.1 Cycle Sequencing kit (Applied Biosystems TM, Thermo Fisher Scientific, Life Technologies Corporation, USA) as per the manufacturer's instructions followed by sequencing under the Genetic Analyser System (Genetic Analysers 3130*xl*, Applied Biosystems). The sequenced data was analysed using MacOsFinchTv version 1.5.0 software.

ELISA for cytokine determination

The levels of IFN-Ɣ, IL-6, IL-10, and IL-22 (Quantikine® ELISA Immunoassay, R&D Systems, Inc. Minneapolis, Minnesota, United States) cytokines in plasma were determined in both study groups using Enzyme Linked Immunosorbent Assay (ELISA) following the manufacturer's instructions.

Statistical analysis

All computations for data analysis were carried out with GraphPad Prism® (Version 8.2.1) software. Data were presented as the mean ± standard deviation (mean ±SD) or mean ± standard error of mean (mean ±SEM). Chi-square goodness of fit was used to verify the agreement of observed genotype frequencies with those expected Hardy-Weinberg equilibria (HWE). Allele and genotype frequencies were compared by standard contingency table analysis using Chi-square test and Odds ratio [95% confidence intervals (CI)] were calculated as an index of the association of the gene with the disease. For the correlation between cytokine concentration and FEV1% (predicted), two-tailed non-parametric

Spearman test was performed. Non-parametric Kruskal-Wallis one-way ANOVA test was used to compare FEV1% (predicted) and genotypes. Whereas, for the correlation between genotypes and cytokine concentration two-way ANOVA (Sidak and Tukey's correction test for multiple comparisons) test was performed. Statistical significance was defined as a p-value <0.05.

Results

The age of AP group was 33.00 ±11.27 (Mean ± SD), which ranged between 20 and 60 years and the age of HC group was 33.29 ±8.71 (Mean ± SD), which ranged between 21 and 58 years (Table 1). The FEV1% (predicted)-pre of patients was 80.97 ±13.27 (Mean ±SD), which ranged between 60 and 106 and FEV1% (predicted)-post was 95.06 ±13.96 (Mean ±SD), which ranged between 73 and 120. The change in FEV1% (predicted) was 18.71 ±5.27 (Mean ±SD), which ranged between 12 and 33. The demographic and clinico-physiological details of the study subjects are given in table 1.

Polymorphism in *CRHR1* gene and their association with asthma

Two SNPs rs242941 and rs242940 were determined in the locus of *CRHR1* gene by the sequence analysis in North Indian population (Fig 1). Correlation between genotypes of rs242941 (Fig. 2A) and rs242940 (Fig. 2B) and FEV1% (predicted) was found to be significantly associated with asthma patients. Genotypic and allelic distributions of SNPs in both study subjects and their association values with asthma are presented in table 2.

Correlation of cytokine levels with asthma

The plasma levels of cytokine IFN-ϒ (p< 0.0001) and IL-6 (p= 0.0003) were significantly increased in asthmatic patients whereas IL-10 (p=0.0028) levels were significantly decreased in asthma patients (Table 3). The levels of IL-22 (p=0.0671) did not increase significantly in asthma patients (Table 3). Correlation between the levels of cytokines IFN- ϒ (p< 0.0001) and IL-6 (p< 0.0001) and FEV1% (predicted) were found to be highly significant and negatively correlated and found positively correlate with IL-10 (p< 0.0001) in asthma patient group (Fig. 3). Whereas, the levels of IL-22 (p= 0.3397) were though found to be negatively correlated with FEV1% (predicated) (but non-significant) in the asthma patient group (Fig. 3).

Correlation between polymorphism in *CRHR1* gene and plasma levels of cytokines

rs242941 vs cytokines concentration

The level of IFN- ϒ was significantly elevated in AA (p= 0.0055) as compared to CC genotype in asthma patient group, and CC (p= 0.0004), CA (p= 0.0003) and AA (p= 0.0075) in asthma patient as compared to healthy control group. Level of IL-6 was found to be significantly elevated in AA (p< 0.0001) genotype as compared to CC genotype and AA (p= 0.0014) genotype as compared to CA genotype in asthma patient group, and AA (p= 0.0010) genotype in asthma patient as compared to healthy control group. A correlation between the concentration of IL-10 and IL-22 and any of the genotypes of *CRHR1* gene was lacking in both study groups. The levels of IL-6 (p= 0.0223) were found to be significantly correlated with the rs242941 genotypes. The details of rs242941 polymorphism and their correlation with the concentrations of various cytokines are given in table 4.

rs242940 vs cytokines concentration

The level of IFN- ϒ was significantly elevated in GG (p= 0.0012), GA (p= 0.0008) and AA (p= 0.0100) in asthma patient as compared to healthy control group. Level of IL-6 was found to be significantly elevated in AA (p= 0.0007) genotype as compared to GG and AA genotype (p= 0.0420) as compared to GA genotype in asthma patient group, and AA (p= 0.0088) genotype in asthma patient as compared to healthy control group. There was no correlation between the concentration of IL-10 and IL-22 and any

genotypes in both the study groups. The details of rs242940 polymorphism and their correlation with the concentrations of various cytokines are given in table 5.

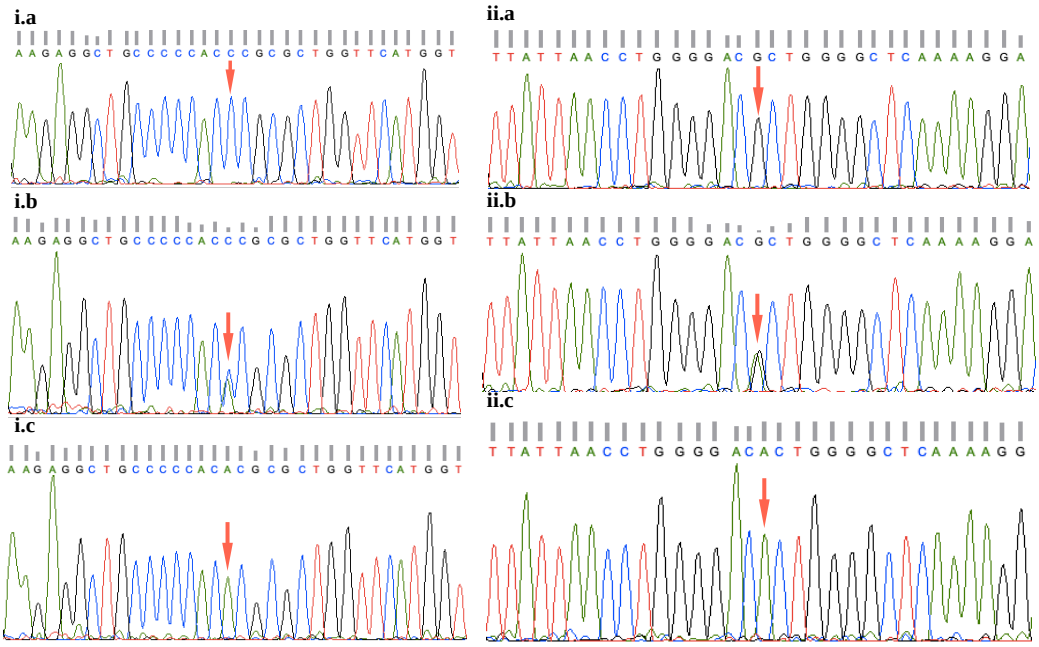

Fig. 1 Chromatograms of *CRHR1* gene polymorphism. i) rs242941 (a) CC, (b) CA, (c) AA, ii) rs242940 (a) GG, (b) GA, (c) AA

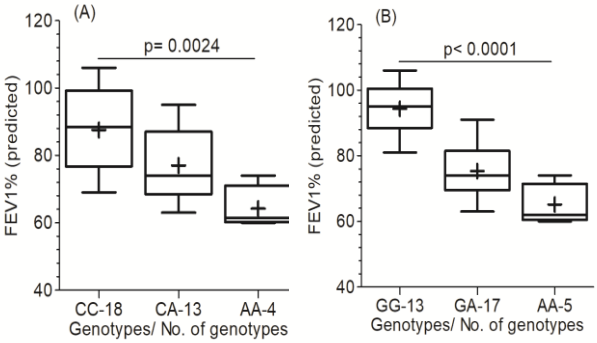

Fig. 2: Boxplots of percent of FEV1 versus genotypes of SNPs. (A) rs242941 and (B) rs242940 (by Kruskal-Wallis test).Horizontal line inside the boxplot is indicating median value and plus (+) sign is indicating mean value of FEV1 % predicted. p< 0.05 was considered as significant.

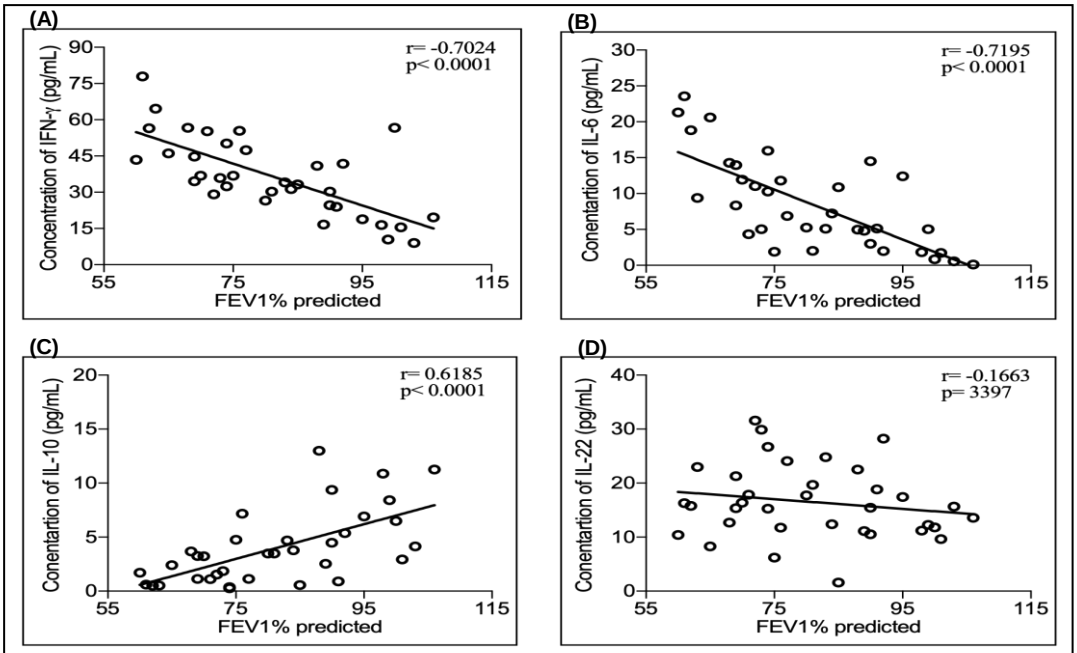

Fig. 3: Correlation between cytokines concentration (pg/mL) (A) IFN-Ɣ, (B) IL-6, (C) IL-10 and (D) IL-22 and FEV1% (predicted) in AP group. $p< 0.05$ was considered as significant.

Table 1: The demographic and clinico-physiological details of study subjects

Sl. No	Subject information parameters		AP (N=35)	HC (N=35)
1	Age (Years) (Mean ± S.D) (Range)		33.00 ± 11.27 (20-60)	32.43 ± 7.78 (21-50)
2	Gender			
	Male		18	19
	Female		17	16
3	Duration of illness		2 months- 15years	NA
4	Family history of asthma			
	Present		14	None
	Absent		21	None
5	Diet			
	Vegetarian		16	18
	Non-vegetarian		19	17
6	Height (Year) (Mean ± S.D) (Range)		158.86 ± 8.56 (142-177)	N.D.
7	Weight (Kg) (Mean ± S.D) (Range)		60.60 ± 11.78 (41-83)	N.D.
8	BMI (Kg/m^2) (Mean ± S.D) (Range)		24.16 ± 4.29 (17.5-31.6)	N.D.
9	FVE1 (Range)	Pre (Mean ± SD)	2.19 ± 0.64 (1.04-3.51)	N.D.
		Post (Mean ± SD)	2.58 ± 0.72 (1.29-3.95)	
		%Pre (Mean ± SD)	80.97 ± 13.27 (60-106)	
		%Post (Mean ± SD)	95.74 ± 13.62 (73-120)	
		% Change (Mean ± SD)	18.71 ± 5.27 (12-33)	
10	FVC (Range)	Pre (Mean ± SD)	3.11 ± 0.83 (1.39-4.99)	N.D.
		Post (Mean ± SD)	3.31 ± 0.84 (1.42-5.03)	
		%Pre (Mean ± SD)	96.29 ± 13.98 (76-129)	
		%Post (Mean ± SD)	102.57 ± 13.68 (81-132)	
		% Change (Mean ± SD)	6.66 ± 5.03 (-2-17)	
11	FEV1/FVC % (Range)	Pre (Mean ± SD)	70.63 ± 8.66 (45-84)	N.D.
		Post (Mean ± SD)	78.07 ± 8.41 (51-92)	
		% Change (Mean ± SD)	10.49 ± 5.29 (1-23)	

N.A.- Not applicable, N.D- Not done, N- Number of study subjects
Age, Height, Weight and BMI: At the time of collection ofblood sample

Table 2: Genotypic distribution and allelic association of CRHR1 gene polymorphism in HC and AP group

SNP details				Genotypes					Alleles								
SNP id	Chromosome no.: Position	Gene position	Gene Region	HC Genotype	AP Genotype	χ^2 test; p value (WHM vs MHT/MHM)	OR	95% CI	Major/ Minor allele	HC Allele	% allele freq. of HC	AP Allele	% allele freq. of AP	χ^2 test; p value	OR	95% CI	MAF
rs242941	17:45815154	30892	Intron 2	CC-22 CA-11 AA-2	CC-18 CA-13 AA-4	0.50; 0.4775 0.98; 0.3218	1.44 2.44	0.49-3.80 0.51-13.73	C>A	C-55 A-15	78.57 21.43	C-49 A-21	70.00 30.00	1.35; 0.2460	1.57	0.74-3.24	.257
rs242940	17:45815234	30972	Intron 2	GG-20 GA-12 AA-3	GG-13 GA-17 AA-5	2.29; 0.1306 1.40; 0.2374	2.18 2.56	0.77-5.82 0.50-10.74	G>A	G-52 A-18	74.29 25.71	G-43 A-27	61.43 38.57	2.65; 0.1034	1.81	0.88-3.67	.321

Wild Homozygote (WHM), Mutant Heterozygote (MHT) and Mutant Homozygote (MHM)
OR (Odd Ratio)- Wild Homozygote (WHM) vs Mutant Heterozygote (MHT), Wild Homozygote (WHM) vs Mutant Homozygote (MHM)
95% CI- 95% Confidence Interval, p< 0.05 considered as significant, MAF- Minor allele frequency

Table 3: Comparison of levels of cytokines and correlation between HC and AP group.

Cytokines	Concentration HC (N=35) (mean ± SEM) (pg/mL)	Concentration of AP (N=35) (mean ± SEM) (pg/mL)	P values
IFN-□	16.24 ± 1.56	36.69 ± 2.73	<0.0001
IL-6	3.71 ± 0.66	8.48 ± 1.08	0.0003
IL-10	6.92 ± 0.77	3.94 ± 0.57	0.0028
IL-22	13.37 ± 1.14	16.50 ± 1.15	0.0671

SEM- Standard error of mean

Table 4: Correlation between genotypes of rs242941 and cytokine concentrations (pg/mL) (mean ± SD) in HC and AP group

		Genotypes			Tukey's multiple comparisons test						Sidak's multiple comparisons test			Two-way ANOVA test		
Cytokines	SG	CC (WT)	CA (MHT)	AA (MHM)	P*1	P*2	P*3	P#1	P#2	P#3	P*#WT	P*#MHT	P*#MHM	P4	P5	P6
IFN-ϒ	HC AP	14.55 ± 9.16 30.60 ± 14.38	19.11 ± 9.71 40.24 ± 14.02	19.05 ± 2.60 52.59 ± 19.56	0.5872	0.8796	>0.9999	0.0902	0.0055	0.1921	0.0004	0.0003	0.0075	0.2765	0.0188	<0.0001
IL-6	HC AP	2.47 ± 3.17 5.81 ± 4.59	6.16 ± 4.41 9.10 ± 5.81	3.89 ± 2.48 18.48 ± 5.81	0.0725	0.9030	0.7866	0.1146	<0.0001	0.0014	0.0637	0.3020	0.0010	0.0223	0.0005	<0.0001
IL-10	HC AP	7.29 ± 5.09 5.34 ± 3.87	6.43 ± 3.88 2.98 ± 1.98	5.43 ± 1.33 0.77 ± 0.64	0.8299	0.8001	0.9409	0.2361	0.0995	0.5932	0.3310	0.1052	0.4447	0.6381	0.1083	0.0141
IL-22	HC AP	12.91 ± 7.21 16.15 ± 7.38	14.27 ± 6.68 16.73 ± 6.49	13.53 ± 1.68 17.31 ± 6.82	0.9958	>0.9999	>0.9999	>0.9999	0.9998	>0.9999	0.3822	0.7753	0.8980	0.9683	0.8530	0.1822

Table 5: Correlation between genotypes of rs242940 and cytokine concentrations (pg/mL) (mean ± SD) in HC and AP group

		Genotypes			Tukey's multiple comparisons test						Sidak's multiple comparisons test			Two-way ANOVA test		
Cytokines	SG	GG (WT)	GA (MHT)	AA (MHM)	P*1	P*2	P*3	P#1	P#2	P#3	P*#WT	P*#MHT	P*#MHM	P4	P5	P6
IFN-ϒ	HC AP	12.98 ± 8.06 29.65 ± 14.35	21.40 ± 9.85 39.59 ± 13.70	17.30 ± 4.66 45.17 ± 23.72	0.1662	0.8447	0.8683	0.0890	0.0561	0.6558	0.0012	0.0008	0.0100	0.5462	0.0133	<0.0001
IL-6	HC Ap	2.22 ± 3.20 5.30 ± 4.39	6.06 ± 4.26 9.01 ± 5.43	4.25 ± 1.58 14.96 ± 9.35	0.0775	0.7711	0.8266	0.0950	0.0007	0.0420	0.2065	0.2839	0.0088	0.1196	0.0016	0.0002
IL-10	HC AP	7.52 ± 5.32 4.58 ± 3.67	6.56 ± 3.55 4.37 ± 3.27	4.35 ± 0.48 0.84 ± 0.58	0.7865	0.4069	0.6664	0.9887	0.1817	0.1963	0.1204	0.3832	0.5452	0.8950	0.1093	0.0181
IL-22	HC AP	12.82 ± 7.59 14.93 ± 7.25	13.85 ± 6.31 17.86 ± 6.63	15.17 ± 1.14 15.95 ± 6.64	0.9990	0.9948	0.9998	0.8276	0.9999	0.9952	0.7761	0.3367	0.9981	0.7973	0.5252	0.2677

SG- study groups, WT- Wild type, MHT- Mutant heterozygote, MHM- Mutant homozygote
[1]WT vs MHT, [2]WT vs MHM, [3]MHT vs MHM, *HC, #AP, [4]Interaction of all genotypes, [5]Interaction of same genotypes in HC and AP, [6]Interaction of different genotypes in HC vs AP

Discussion

Many of the previous studies suggested the involvement of the various Th cell cytokines in the development and regulation of inflammation in asthma[13]. Studies also suggested the possible role of HPA axis to maintain the homeostasis of various Th cell cytokines in the inflammatory response in asthma. In this study, the correlation between *CRHR1* gene polymorphism and the levels of Th cell cytokines were determined in asthma in North Indian population. The frequency of genotypes and alleles of SNPs which were found in the sequenced region of the *CRHR1* gene were not significantly associated with asthma. The expression study of cytokines may suggest their role in the development, exacerbation and severity of inflammation in asthma and establish the evidence in favour of some previous studies[16-18].

However, our study shows the contradiction with some earlier studies about theexpression of cytokines in asthma[18].

In our study, the level of IFN- ϒ and IL-6 were found significantly elevated in AP as compared to HC group and significantly and negatively correlated with FEV1% (predicated). IFN- ϒ is released by Th1 lymphocytes, and both elevated and non-elevated serum levels have been reported in asthmatic patients[19-21]. The results show AA homozygote mutant genotype of rs242941 is associated with a higher level of IFN- ϒ in AP group. Whereas, intergroup comparison of IFN- ϒ level shows the higher expression in AP group as compared to HC group in all corresponding genotypes of both SNPs. IL-6 is a well-known pro-inflammatory cytokine present in circulating blood as well as bronchoalveolar lavage fluid in asthma[22-24]. This study supports the previous studies and also suggest the association between *CRHR1* gene polymorphism and IL-6 levels. The heterozygote and homozygote mutant genotypes of both SNPs show the higher level of IL-6 expression as compared to wild type genotypes in AP group. In intergroup analysis, only AA genotype shows higher level of IL-6 expression in AP group as compared to HC group. The results of study suggest that the mutant homozygote genotypes of both SNPs might be associated with higher levels of both inflammatory cytokines. In this study, IL-10 levels were found significantly decreased in AP as compared to HC group and significantly and positively correlated with FEV1% predicted. IL-10 is mainly secreted by Treg cells as a potent regulator of inflammatory responses and plays a critical role in controlling allergic airway inflammation[25-27]. IL-10 inhibits the production of IFN- ϒ by Th1 lymphocytes[28]. Therefore, Low level of IL-10 may be associated with poor control of asthma. Reduced IL-10 production increases the production of pro-inflammatory cytokines, leading to chronic inflammation, airway remodelling, airflow limitation, and lung tissue damage[29]. IL-22 is produced by Th22 cells[30,31]. Studies reported both pro and anti-inflammatory role of IL-22 in airway inflammation[32-34]. IL-22 levels were increased non significantly in AP as compared to HC. A correlation between the concentration of IL-10 and IL-22 and any of the genotype of SNPs in *CRHR1* gene was lacking. The expression of both the cytokines is likely to have none of the association with both SNPs of *CRHR1* gene.

Conclusion

The allele frequencies of SNPs in *CRHR1* gene are not associated with asthma. Still, the genotypes of SNPs are found to be significantly correlated with IFN- ϒ and IL-6 cytokines in North Indian population. We also found the significant correlation between FEV1% (predicted) and IFN-ϒ, IL-6 and IL-10 concentrations as severity markers for asthma.

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Conflict of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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