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Non-significant association of IL-4 gene polymorphism with Asthma in Vindhyan population (India)

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Abstract

Interleukin-4 (IL-4) intervenes essential pro-inflammatory functions in asthma including enlistment of the IgE isotype switch, expression of vascular cell adhesion molecule-1 (VCAM-1), advancement of eosinophil transmigration crosswise over endothelium, mucus secretion and differentiation of T helper type 2 lymphocytes leading to cytokine release. Asthma is a complex hereditary confusion that has been connected to polymorphisms in theIL-4 gene promoter and proteins involved in IL-4 signaling. Soluble recombinant IL-4 receptor needs transmembrane and cytoplasmic initiating spaces and can in this manner sequester IL-4 without interceding cell actuation. We report the consequences of beginning clinical preliminaries, which show clinical viability of this normally happening IL-4 rival as a remedial specialist in asthma. In asthmatic case lower number of physical dynamic people was viewed when contrasted with control (41.57% Vs 53.33 %) a noteworthy relationship of physical movement was seen (Chi square esteem 5.524, df-1 and P esteem 0.0188). Bidi and cigarette smokers were incorporated as smoking populace. The information shows the level of smokers was not particularly extraordinary between both in the event that and control populace (25.79% Vs 22.38%) and no noteworthy contrast was seen. Information from polymorphic screening, Non-huge dimension of progress has been seen in dispersion of IL-4 genotypes in Healthy Control (HC) amass when contrasted with asthmatic patients gathering in spite of the fact that HC group indicated little increment in like manner 'B1/B1' genotype when contrasted with Patients of asthma(5.62% versus 5.3% individually) yet contrast was difference was nominal and statistically non- significant. Likewise, 'B2/B2' genotype was available at lesser recurrence in asthmatic patients aggregate 51.9% and furthermore in charge gather 54.2%. The general genotype was measurably non-significant ($\chi 2$ =0.1918, P=0.9086) however 'B1/B1' genotype recurrence was higher in control gathering and might be defensive in our populace.

Key- words: Asthma, Genotype, Allele, interleukin-4 , T helper lymphocytes, PCR

Introduction

Bronchial asthma is the most common chronic diseasebamong children in the world. It affects up to 300 million people worldwide. Bronchial asthma is influenced by genetic and environmental factors. The disease is defined by the presence of airway hyper-reactivity, mucus overproduction, and chronic eosinophilic inflammation. Asthma is often characterized by enhanced total serum IgE level upon the exposure to allergens, which is known as an atopy. Many family studies, through genome-wide linkage studies, confirmed the involvement of genetic predisposition in the development of atopy in asthmatic patients. The elevated IgE production in asthmatic patients results in promotion of acute hypersensitivity responses, chronic eosinophil-predominant allergic inflammation with T helper-2 (Th2) cells cytokine production [1].

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IL-4 serves as an essential proinflammatory cytokine in immune regulation mediated by activated T helper cells (Th) and facilitates immunoglobulin E isotype switching in B cells, growth, and differentiation of B cells and monocytes. As an important signal molecule, IL-4 can exacerbate airway inflammation through modulating eosinophils, lymphocytes, and air epithelial cells that play an important role in the pathogenesis of asthma. Moreover, IL-4 plays a pivotal role in phenotypic changes of bronchial asthma, such as airway hyperresponsiveness, eosinophil infiltration, and mucus overproduction. Also STAT6, the signaling molecule from JAK/STAT pathway, activated by IL-4 and IL-13 cytokines, plays an important role in IgE production and allergic airway inflammation [1-3].

The Th2 cytokines [interleukin (IL)-4, IL-5, IL-9, IL-10, and IL-13] have a substantial effect on the pathogenesis of atopic diseases. IL-4 is produced by activated T cells, mast cells, and basophils, which

participate in IgE synthesis and Th2 phenotype differentiation in T cells, and play a crucial role in the development of atopic diseases including asthma [4-8]. Genome-wide association studies have suggested that an asthma susceptible locus could be mapped to chromosomes 5q31-33, covering genes encoding for human IL-4, IL-13, and IL-16. Moreover, the IL-4 gene was associated with asthma in more than three independent study populations. A polymorphism (C-589T) in the IL-4 promoter region seems to be related to elevated serum levels of IgE. Another single nucleotide polymorphism (SNP), C-33T, located in the 5'-untranslated region (UTR) of IL-4, was reported to be associated with asthma. Additionally, although a third polymorphism, G-1098T, has been investigated in several cohorts, its correlation with asthma varies across studies. Further investigations indicated that the polymorphisms C-589T and C-33T were associated with asthma by some authors, but by no means all, which may be due to small sample sizes, low statistical power, and/or clinical heterogeneity. Due to these conflicting and controversial results, combined evidence needs to be taken into account in order to assess the association between IL-4 promoter polymorphisms and asthma. In the present study, we carried out a meta-analysis to evaluate the association of IL-4 promoter polymorphisms with asthma susceptibility risk among different ethnic and asthma status groups [11-16].

Material and Methods

Life Style Factors:

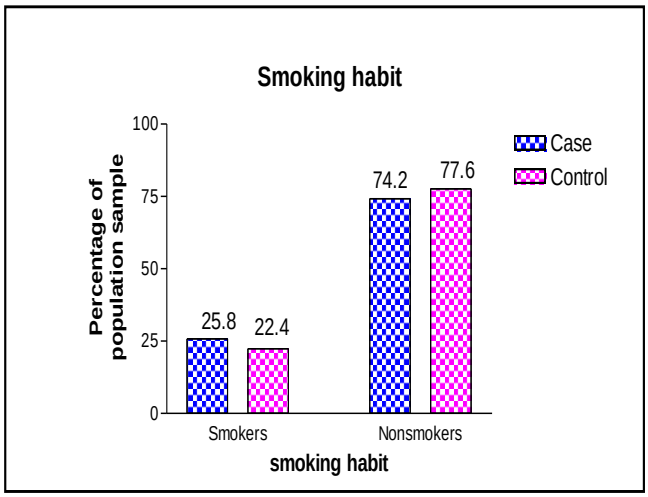
Physical activity and smoking habits were asked to both case and control individuals during collection of samples by a brief questionnaire and the data obtained are depicted in table no. 1. Physical activity was decided on the basis of CDC 2010 recommendations and criteria. In asthmatic case lower number of physical active persons was seen as compared to control. (41.57% Vs 53.33 %) a significant association of physical activity was seen (Chi square value 5.524, df- 1 and P value 0.0188). An odds ratio of physical activity was 0.6227 (CI =0.4191-0.9253) which indicates the positive association of physical activity with prevention of asthma and active life style could be concluded as a very important life style factor which can prevent pathophysiology of asthma. Inactive lifestyle could be important factor which could increase the prevalence of asthma pathophysiology **[21-22].**

Smoking habit data were also collected during questionnaire organized during sample collection and the data obtained are depicted in table no.1. Here mixed urban as well as rural population were included in present investigation and we did not discriminated light and heavy smokers because of irregular smoking quantity of patients. Bidi and cigarette smokers were included as smoking population. The data indicates the percentage of smokers was not very much different between both in case and control population (25.79% Vs 22.38%) and not any significant difference was seen. Odds ratio of smokers were 1.205 which indicates that possibly smoking could be a risk factor associated with asthma pathophysiology of asthma although the mode of effects are not very much clear**.**

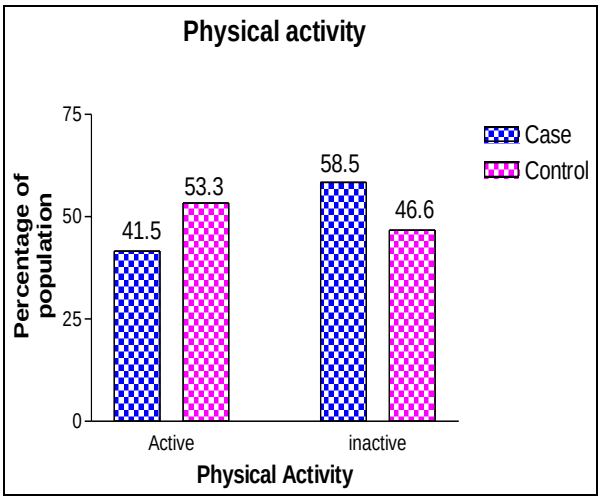
Table 1: Life Style Factors selected in present investigation

Life style factors	CASE N= 190 n %	CONTROL N=210 n %	Chi square value χ^2 (P Value)	ODDS RATIO AND CI
Physical active population (according to CDC 2010)	79 41.57 %	112 53.33	5.524 (0.0188 *)	0.6227 (0.4191 - 0.9253)
Smoking Habits **Light and heavy smokers**	49 25.79	47 22.38	0.6354 (0.4254)	1.205 (0.7613 - 1.908)

N – Number of individuals in study group
%- Genotype allele frequency and carriage rate expressed in percentage
* denotes the significant level of association between case and control



Graph No. 1: Percentage of smoking habit in the population



Graph No. 2: Percentage of physical activity in the population

StudyPopulation

The asthmatic patients taking an interest in this investigation were from Asthma and Allergy Department, The Gandhi Medical hospital, Rewa. Our researched people contains 450 people that join 160 asthma patients and 190 sound masses of Vindhya locale (Madhya Pradesh). In this examination , we assembled blood test from the asthma patients who went to the Medicine division of Shyam Shah Medical school and Sanjay Gandhi Memorial Hospital (SGMH) Rewa . Moreover Regional accumulations purpose of assembly of Ranbaxy pathology, Rewa , region emergency clinic , Sidhi ,Satna just as solid masses in this examination.

Sample Collection

Blood tests were drawn by venipuncture of the cubital vein from every person. All examples were taken somewhere in the range of 8 and 10 am (morning) as they were to be broke down for adrenal capacity. Their blood levels top somewhere in the range of 8 and 10 toward the beginning of the day achieving its most minimal dimension by night.

Five mL blood was gathered in vails containing EDTA as entire blood for secluding leukocytes and extraction of DNA. For serum, 5 mL was taken in a separate cylinder, continued remaining in a cylinder for a couple of minutes and centrifuged (Hettich, Germany) at 14,000 x g for 10 minutes and serum was isolated. Three aliquots were produced using every entire blood and serum test and put away at -80 °C till use.

Genotyping of IL -4 Polymorphisms

Method for DNA isolation

Genomic DNA was removed from entire blood by the adjustment of salting out technique depicted by Miller and colleagues (Miller et al. 1988).Frozen blood test was defrosted at room temperature. 0.5 ml of entire blood test was suspended in 1.0ml of lysis buffer (0.32M sucrose, 1mM Mgcl2, 12 mM Tris,and 1% Triton X 100) in a 1.5ml microcentrifuge tubes. This blends blended delicately by modifying the vial topsy turvy (upside down) for 1 min. The blend was then permitted to represent 10 min at room temperature to guarantee legitimate lysis of cells.The blend was centrifuged at 11000 rpm for 5 min at 4 °C to pallet the nuclie. The supernatant was disposed of cautiously in a container containing disinfectant, as pallet formed is losely holds fast(adheres) to the base of rotator tube. The pallet was resuspended in 0.2 ml of lysis support and recentrifuged at 11000 RPM for 5 minutes. The pallet was then dissoved in 0.2 ml of deionised autoclaved water and blended completely on vortexer. The blend was recentrifuged at 14000 RPM for 1 min at 4 °C. Supernatent was disposed of to pick up an intaccr bed. To the above pallet, 80 micro litre of proteinase K buffer (0.375 M NaCl, 0.12 M EDTA, pH 8.0) and 10 micro litre of 10% SDS (10% w/v SDS, pH 7.2) was included. Blend was whorl foamed with the assistance of micro tip to permit appropriate lysis of pallet nuclei.

After the total absorption , 100 micro liter of soaked cold 5 M NaCl was included and shaken vigorously for 15 seconds. To the above blend 0.2 ml of de-ionized autoclave water and 0.4 ml of Phenol-chloroform (4:2 v/v) were added to expel the greater

part of the non-nucleic corrosive natural molecules. Micro centrifuge rotatory tube was reversed upside down until the arrangement turned smooth. Phages were isolated by centrifuging the above blend at 12000 RPM for 10 min at 4°C. Watery (aqueous) top layer was spared and moved into another micro centrifuge tube.Transferring of any interface layer was avoided. To the aqueous layer, 1 ml chilled absolute ethanol was added and the tube was inverted several times untill the DNA was precipitated. DNA was precipitated like thread. This precipitated DNA was centrifuged at 14000 RPM for 4 min at 4^0C to pallet the DNA thread. Supernatent was then discarded. The pallet was washed twice with 1 ml of 70% alcohol. At that point the blend was again centrifuged at 14000 RPM for 1 min 4°C. Supernantent was again disposed of and pallet was air dried for 10-20 mins. The pallet DNA was rehydrated in 100-200 miniaturized scale litre of TE buffer, pH-7.4 (10 mM Tris-HCl pH-7.4, 1 mM EDTA, pH-8.0) at that point DNA was permitted to broke down overnight at 37°C before quantitating.

Determination of Quality and Quantity of isolated DNA

The isolated DNA is to be utilized for PCR . In this manner its appropriateness for PCR alongside its size, heterogeneity is among the most vital criteria for purity. All DNA arrangements were tested for quality and quantity measures as depicted in the following passage.

Quantitation by UV spectrophotometry

The separated genomic DNAs were then tried for virtue by estimating their absorbance esteems at 230 nm, 260 nm, 280 nm and 300 nm and chilled utilizing an UV visible spectrophotometer (Systronic, India). A DNA readiness was viewed as great on the off chance that it had a 260 nm/280 nm proportion as around 1.8 and A 300 nm was 0.1 or lesser. The absorbance at 260 nm was utilized to figure the measure of DNA, utilizing the relationship that twofold standard DNA at 50 microgram/ml focus has an absorbance = 1.0 at 260 nm.

Agarose Gel Electrophoresis

Gel electrophoresis of the genomic DNA was done for qualitative estimation of tests arranged. A decent DNA readiness shows up as single band. A horizontal agarose piece gel electrophoresis mechanical assembly (Bangalore Genei, Bangalore, India) was utilized. To sum things up, 2 small scale liter of each genomic DNA was stacked on 0.8 agarose (0.8% w/v, Sigma) containing Ethidium Bromide arrangement (0.5 smaller scale gram/ml) and electrophoresis was done at 80 V in 1X TAE cushion (40 mM Tris, 20 mM Acetic, 1 mM EDTA). Lambda DNA Eco R1/Hind III twofold summary (Bangalore, Genei, Bangalore, India) was utilized as molecular weight marker after culmination of electrophoresis, the DNA groups were envisioned and captured utilizing an UV trans illuminator (312 nm) and Gel documentation framework (VilberLourmate, Cedex 1, France) separately.

Quantification of extracted DNA

Genomic DNA from test were then assessed for the perfection (abstract and Quantitative investigation)by evaluating their absorbance (A°) values at 230 nm , 260 nm ,280 nm, and 300nm using Nanodrop 2000 (Thermo Scientific).In the occasion that the DNA had absorbance at 260 nm/280nm. Extent as round 1.8 A°and 300 nm was 0.1 or lesser then DNA arranging was seen as high - quality and quantity.

Polymorphism Screening

Genomic DNA extricated from fringe blood of healthy people and ailing(diseased) people were exposed to PCR pursued by restriction digestion and electrophoresis to genotype both the gatherings for applicable quality of intrigue. All the PCRs were done in a PTC 200 thermo cycler (MJ Research Inc, USA). PCR is a quick, cheap and straightforward methods for creating moderately huge duplicate number of DNA molecule from the little measures of source DNA material, notwithstanding when the source DNA is of generally low quality. Because of the extreme sensitivity, precautions were taken against contamination of the reaction mixture with the follow measure of DNA, which could serve in as an undesirable template. Appropriate negative control was incorporated into each PCR run did for every quality, to screen disinfecting of PCR mix to maintain a strategic distance from any bogus positive outcomes. The negative control utilized for PCR contained entire PCR response blend with the exception of target DNA, which was supplanted by HPLC sanitized water, free of RNAs, DNAs and any contamination from some other source, looking like the quality grouping **[11].**

Therefore restriction enzyme digestion was performed by incubating the double standard DNA with suitable measure of restriction enzyme in its respective buffer as suggested by the provider and at ideal temperature for that particular chemical. A typical digestion incorporates one unit of protein for every micro gram of beginning DNA. One enzyme unit is generally characterized as the measure of enzyme expected to totally process one micro gram of double standard DNA stranded in one hour at the suitable temperature. There bio synthetic action of

the restriction enzyme is the hydrolysis of phosphodiester spine at specific destinations in a DNA grouping. Precautionary measure was taken to maintain a strategic distance from star action of restriction enzyme. At the point when DNA is processed with certain restriction enzymes under non-standard condition, cleavage can happen at locales distinctive at recognition sequences. Such atypical cutting is designated "Star Activity" which can be because of high pH (>8.0) or low ionic quality, incredibly high pH enzyme(>100 U/microgram of DNA) and presence of organic solvents in the reaction (for example Ethanol, DMSO). The PCR and restriction digestion processing conditions were advanced for explicit locus of a pertinent fragment of the quality to be contemplated. The PCR products and the digested products were isolated on either agarose gel or polyacrylamide gel, contingent upon their size. Gels were stained with ethidium bromide arrangement (0.5 microgram/ml) and therefore pictured and shot under UV transilluminator.

Detection of interlukin-4 (IL-4) via PCR- RFLP:

Primer

The oligonucleotides sequences (primers) used were the sequences Flanking this regions were described by (Chaudhary AG, 2008) They are as follow:

IL-13 forward primer: 5′-ACA CAC CAT CCT GCC CCA G-3′;

IL-13reverse primer: 5′-TAC CCC CTC CAG AGA GCA GG-3′)

PCR Mix

The PCR was completed in a final volume of 25 µl, containing :100mg of genomic DNA(4-5 µl) from the sample, then 2.5 µl of 10X support (10 mM Tris HCl pH 8.8, 50 mM KCl, 1.5 mM MgCl_2, 0.01% gelatin, 0.005% NP 40; last concentration1X; Genetix Biotech Asia pvt. Ltd., India) then 1µl of 10mM dNTPs (BangloreGeneie , Banglore, india), then 1µl of 25pmol/µl of forward and reverse primers specific for IL-1β. then0.2 µlTaq DNA polymerase(5U/µl)in final concentration 1 U (BangloreGeneie Asia Pvt .Ltd ,India) and sterile water to set the volume of response blend to 25 µl.

Thermal Profile

Thermal Profile utilized for the amplification of wanted portion of gene was as per the following: Initial denaturation at 95°C for 2 min and 35 cycles of Denaturation at 95°C for 1 min , annealing at 54°C for 1 min and extension at 74°C for 1 min , pursued by final extension at 74°C for 10 min . PCR items were isolated on 2% w/v, Sigma) utilizing a 100 bp atomic weight (MW) marker to affirm the PCR item size of 304 bp.

Restriction Digestion by AvaI

The C to T transition in promotor region of IL-1β quality when amplified by PCR was than incubated with AvaI restriction enzyme(new England Biolabs ,USA).The reaction mixb included 0.3µl of 10,000µ/ml AvaI restriction enzyme (final concentration 3U),2.0µl of 10 X NEBuffer 4 (last fixation 1 X ; 50 mM potassium acetic acid derivation, 20 mM Tris acetic acid derivation , 10 mM magnesium acetic acid derivation , 1 mM DTT , pH 7.9), 12.0 µl of PCR item and 5.7 µl of sterile water. Reaction was incubated for 16 hrs at 37°C for complete digestion 10 µl of digested PCR item was stacked on 9% polyacrylamide gel. Electrophoresis was done at 80 V in 1 X Tris - borate EDTA buffer (89 mM Tris pH 7.6, 89 mM boric corrosive , 2 mm EDTA pH 8.0). A 100 bp quality DNA ladder(Roche, Germany) was run simultaneously as atomic weight marker . The items were pictured utilizing a UV transilluminator . The gel picture was caught utilizing digital camera and a gel documentation programming (VilberLourmate , Cedex France).

Genotyping of IL-4

Digestion of the amplified 253bp PCR item gave two fragments of 183bp. and, 70bp respectively if the product was excisable by Ava I . Contingent upon processing pattern product were scored as genotype CC when homozygous for presence of Ava I site genotype TT when homozygous for absence of Ava I site and genotype CT if there should be an occurrence of heterozygosity.

Statistical Analysis

Statistical analysis was finished by looking at the dispersion of genotype frequencies, allele frequencies and carriage rates of all the three polymorphism in ailing and control gathering. The extent of an allele in an example will be the proportion of number of events of the examined allele in the populace to the all out people conveying something like one duplicate of test allele separated by the all out number of people. Information was dissected utilizing Microsoft Excel 2002, Microsoft Corporation.

Results and Discussion

Detection of Genetic Polymorphism in IL-4

An anti-inflammatory cytokine IL-4 cytokine regulates the inflammation by increasing the production of many associated anti-inflammatory cytokines such as IL10, IL1RN. The VNTR polymorphism has been seen in intron 3 of IL4 gene. 70 bp VNTR polymorphism was analyzed by PCR.

Two different alleles have been found after PCR. First one is 183 bp containing two VNTR and second one is 253 bp containing three VNTR (figure no.1).

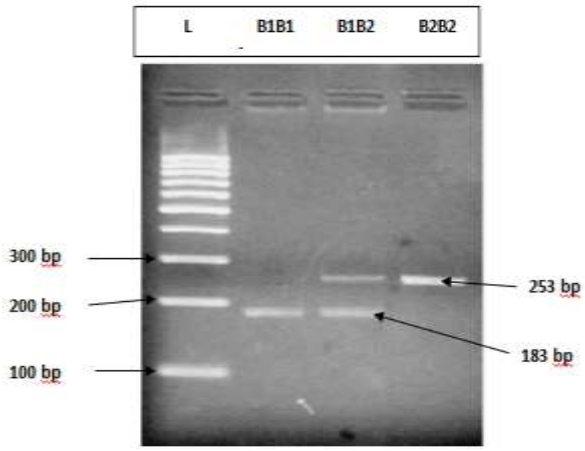


Fig. 1: Representative gel picture of IL-4 VNTR polymorphism restriction digestion
IL-4 third intron polymorphism PCR products. Lane 1: 100 bp DNA marker (Bangalore genei); Lane 2: Homozygous B1B1 (183 bp); Lane 3: Heterozygous B1B2(183 bp and 253 bp); Lane 4: Homozygous B2B2 (253 bp)

The distribution of the polymorphism of IL-4 was consistent with Hardy- Weinberg equilibrium (HWE) in asthma patients as well as in healthy controls. The observed genotype frequencies, allele frequencies and carriage rates for IL-4 is depicted in Table no.2 and Table no.3 and Graph 3, 4, and 5. Non-significant level of change has been observed in distribution of IL-4 genotypes in Healthy Control (HC) group as compared to asthmatic patients group although HC group showed little increase in common 'B1/B1' genotype as compared to Patients of asthma(5.62% *vs.* 5.3% respectively) but difference was nominal and statistically non- significant. Similarly, 'B2/B2' genotype was present at lesser frequency in asthmatic patients group 51.9% and also in control group 54.2%. The overall genotype was statistically nonsignificant (χ^2 =0.1918, P=0.9086) but '*B1/B1'* genotype frequency was higher in control group and may be protective in our population. The odds ratio of B1/B1 genotype was 1.073 which indicates little protective effect whereas an odds ratio of B1/B2 genotype is 1.085 of asthmatic patients group respectively indicate little or no effect and association of this mutant genotype with the asthma susceptibility.

Major allele 'B1' was found at slightly lower frequency in asthmatic group (26.9%) as compared to HC group (25.5%) whereas allele '*B2'* was present in slightly higher frequency in the disease group (73.1% in patients and 74.5% in control) but the difference was nominal and not statistically significant (χ^2 = 0.1636, P=0.6858). An odds ratio of rare allele 'B2' shows moderate effect of minor allele in asthma susceptibility. Carriage rate of allele 'B2' was slightly higher in asthmatic group as compared to healthy control (94.7% Vs 94.4%) whereas carriage rate of allele '*B1*' was approximately similar in both control and disease group and no significant level of change has been seen. Odds ratio of minor allele B2 carriage is 0.9478 which did not suggest any association of B2 allele carriage with disease susceptibility. The pattern of genotype distribution, allele frequency and carriage rate in disease and control group suggests IL-4 is not significantly associated with asthma in our population.

Table 2: Frequency distribution and association of Genotype, allele frequency and carriage rate of IL4 allele (70 bp VNTR) in population of Vindhyan region using Chi Square Test

IL4 GENOTYPE	CASE N= 160		CONTROL N=190		CHI SQUARE VALUE
	n	%	N	%	χ^2 (P Value)
B1B1	9	05.62	10	5.3	
B1B2	68	42.5	77	40.5	0.1918 (0.9086)
B2B2	83	51.9	103	54.2	
ALLELES					
B1	86	26.9	97	25.5	
B2	234	73.1	283	74.5	0.1636(0.6858)
CARRIGE RATE					
B1					
B2	77	48.1	87	45.8	0.0783 (0.7796)
	151	94.4	180	94.7	

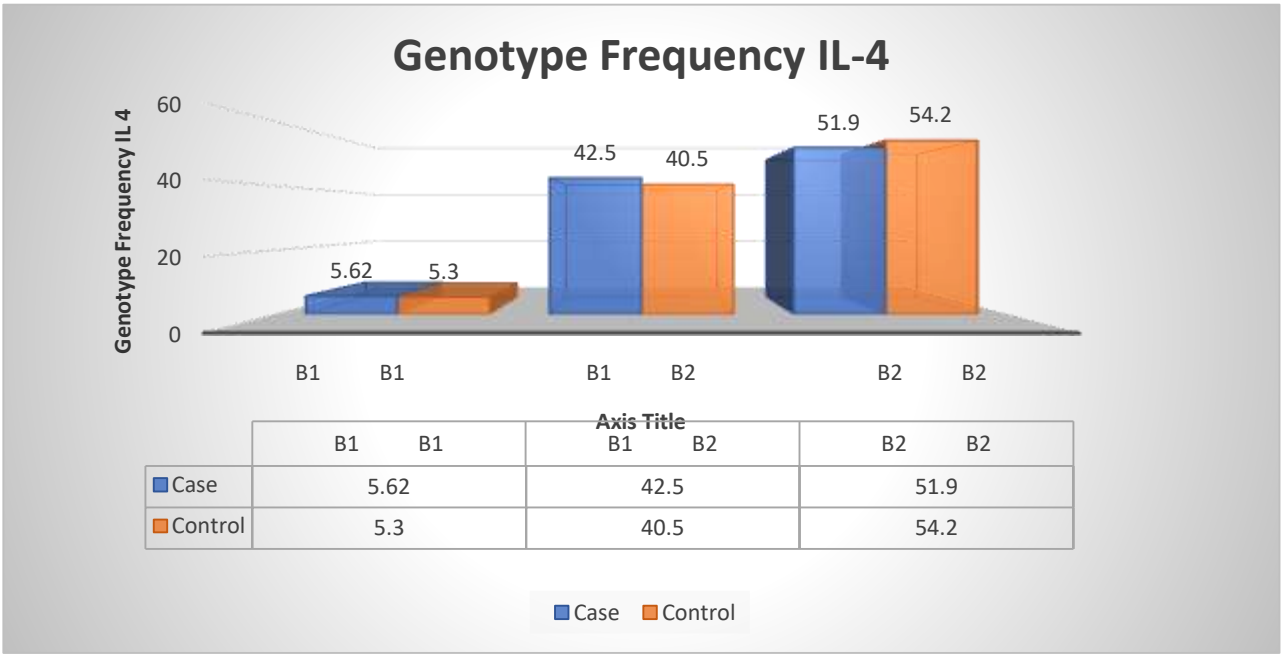
N – Number of individuals in study group

%- Genotype allele frequency and carriage rate expressed in percentage
* denotes the level of significant association between case and control

Table No. 3: Fisher Exact Test values of IL4 VNTR polymorphism

IL-4 GENOTYPE	CASE N= 160		CONTROL N=190		P Value	ODDS RATIO (95% CI)
	N	%	N	%		
B1B1	9	5.62	10	5.3	1.000	1.073 (0.4248 – 2.709)
B1B2	68	42.5	77	40.5	0.7444	1.085 (0.7078 – 1.662)
B2B2	83	51.9	103	54.2	0.6688	0.9105 (0.5973 – 1.388)
ALLELES						
B1	86	26.9	97	25.5	0.7300	1.072 (0.7646 – 1.504)
B2	234	73.1	283	74.5		0.9326 (0.6651 - 1.308)
CARRIGE RATE						
B1	77	48.1	87	45.8	0.8481	1.055 (0.7249 - 1.536)
B2	151	94.4	180	94.7		0.9478 (0.6512 - 1.380)

(* denotes the level of significant association between case and control)
N – Number of individuals in study group
%- Genotype allele frequency and carriage rate expressed in percentage



Graph No. 3: Genotype frequency of IL4

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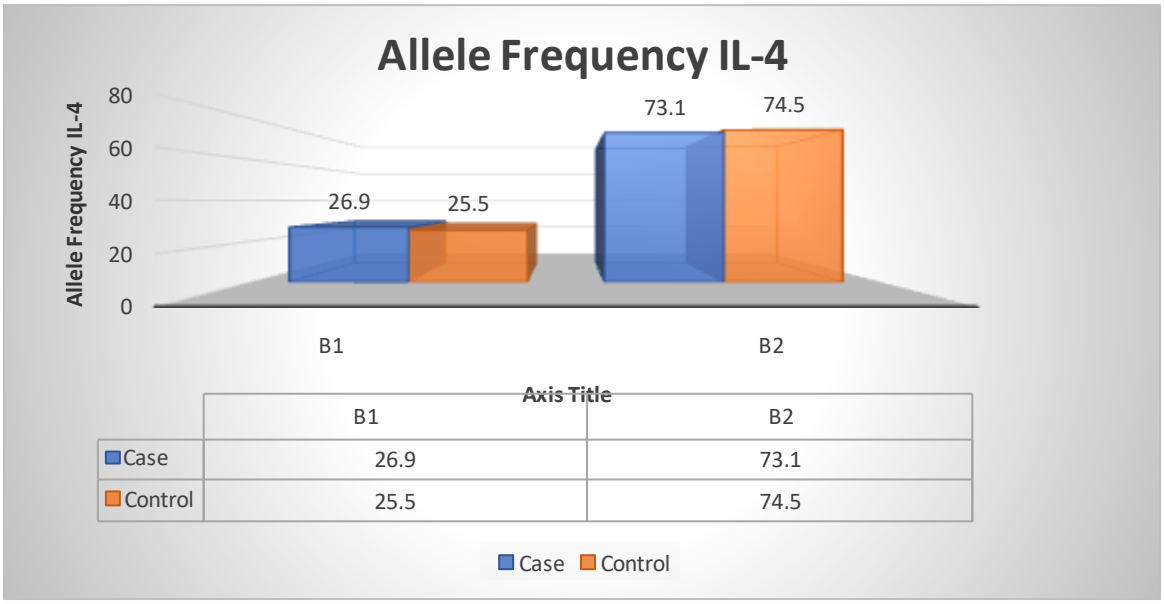
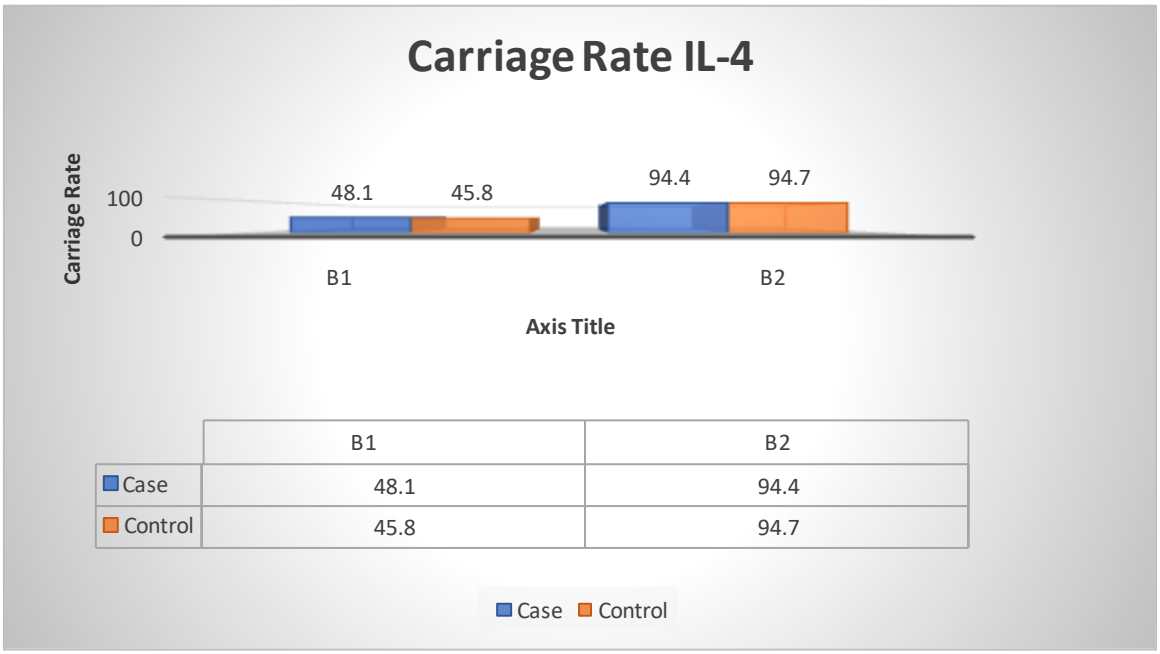


Graph No. 4: Allele frequency of IL4



Graph No. 5: Carriage Rate of IL4

Conclusion

Asthma is a chronic disease that affects the airways of your lungs. Your airways are the breathing tubes that carry air in and out of your lungs. When you have asthma, your airways become swollen. This swelling (inflammation) causes the airways to make thick, sticky secretions called mucus. Asthma also causes the muscles in and around your airways to get very tight or constrict. This swelling, mucus, and tight muscles can make your airways narrower than normal and it becomes very hard for you to get air into and out of your lungs. [2,4].

Asthmatic case lower number of physical active persons was seen as compared to control (41.57% Vs

53.33 %) a significant association of physical activity was seen (Chi square value 5.524, df- 1 and P value 0.0188). An odds ratio of physical activity was 0.6227 (CI =0.4191-0.9253) which indicates the positive association of physical activity with prevention of asthma and active life style could be concluded as a very important life style factor which can prevent pathophysiology of asthma. Bidi and cigarette smokers were included as smoking population. The data indicates the percentage of smokers was not very much different between both in case and control population (25.79% Vs 22.38%) and not any significant difference was seen. Odds ratio of smokers were 1.205 which indicates that possibly smoking could be a risk factor associated with asthma pathophysiology of asthma [22].

Interleukin-4 (IL-4) mediates important pro-inflammatory functions in asthma including induction of the IgE isotype switch, expression of vascular cell adhesion molecule-1 (VCAM-1), promotion of eosinophil transmigration across endothelium, mucus secretion, and differentiation of T helper type 2 lymphocytes leading to cytokine release. Asthma is a complex genetic disorder that has been linked to polymorphisms in the IL-4 gene promoter and proteins involved in IL-4 signaling [10].

Our study of IL4 gene polymorphism reveals 'B1/B1' genotype as compared to Patients of asthma(5.62% *vs. 5.3*% respectively) but difference was nominal and statistically non- significant. Similarly, 'B2/B2' genotype was present at lesser frequency in asthmatic patients group 51.9% and also in control group 54.2%. The overall genotype was statistically non significant (χ^2 =0.1918, P=0.9086) but '*B1/B1'* genotype frequency was higher in control group and may be protective in our population.

Our statistical data suggest, odds ratio of B1/B1 genotype was 1.073 which indicates little protective effect whereas an odds ratio of B1/B2 genotype is 1.085 of asthmatic patients group respectively indicate little or no effect and association of this mutant genotype with the asthma susceptibility. allele 'B1' was found at slightly lower frequency in asthmatic group (26.9%) as compared to HC group (25.5%) whereas allele '*B2'* was present in slightly higher frequency in the disease group (73.1% in patients and 74.5% in control) but the difference was nominal and not statistically significant (χ^2 = 0.1636, P=0.6858). An odds ratio of rare allele 'B2' shows moderate effect of minor allele in asthma susceptibility. Carriage rate of allele 'B2' was slightly higher in asthmatic group as compared to healthy control (94.7% Vs 94.4%) whereas carriage rate of allele '*B1*' was approximately similar in both control and disease group and no significant level of change has been seen. Odds ratio of minor allele B2 carriage is 0.9478 which did not suggest any association of B2 allele carriage with disease susceptibility.

A number of studies of genetic epidemiology have assessed the association of C-589T (also referred to as C-590T; rs number, 2243250) polymorphisms in the promoter region of interleukin-4 (IL-4) gene with asthma in different populations. *Lia T, et. al.* performed a meta-analysis of the association between C-589T polymorphisms of IL-4 and asthma. This meta-analysis suggests there may be an important effect of single nucleotide polymorphisms (SNPs) in the promoter region of IL-4 gene on the pathogenesis of atopic asthma and likewise our study IL4 gene polymorphism are not associated with Asthma [11].

Another study of Nagarkatti R. et. al. is not support to our study. His case-control study was to determine the association between *IL4* and asthma in North Indians. The proximal promoter region of the *IL4* gene was found to be invariant. Previously reported polymorphisms, –590 C/T and +33 C/T, were found to be absent in our population. The χ^2 test using only large expected cell counts (more than 5% of the sample size) showed a significant association between allele size and disease status (χ^2 = 38.08, d.f. = 6, $p < 0.05$). In addition, a significant difference was observed for the allele and genotype frequencies ($p < 0.0005$ and $p = 0.0009$, respectively) in the patient and the control groups. His studies indicate that the promoter of the *IL4* gene is invariant in our population. The case-control studies on the CA repeat polymorphism in the 2nd intron of the *IL4* gene have shown interesting results and indicate the need for further family-based studies [12].

The study of Rad I A, et. al. was similar to our findings. there were any differences in IFN-γ +874 (A/T) and IL-4 -590 (C/T) single nucleotide variations in asthmatic patients compared to normal controls among West Azerbaijani population. The allele or genotype frequencies of IFN-γ +874 A/T in patients were not different from those of controls ($p>0.05$). The differences between allelic or genotypic frequencies of IL-4 -590 C/T in patients and controls were not statistically significant ($p>0.05$).These findings showed that IL-4 -590 (C/T) and IFN-γ +874 (A/T) polymorphisms were not associated with asthma susceptibility [13].

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