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Research Paper

Genetic polymorphism of *FOXO3a (*rs13217795) gene and its association with Asthma

Parwat Raj Pandey[1] and Rashmi Arnold[2]
1: Centre for Biotechnology Studies, APS University, Rewa (M.P.)
2: Department of Botany, Govt. Model Science College Rewa (M.P.)

ARTICLE DETAILS

Corresponding Author:
Parwat Raj Pandey

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ABSTRACT

Asthma is a chronic inflammatory disorder delineated by a heightened immunological response due to environmental or genetic factors. Single nucleotide polymorphism studies have shown that FOXO3a plays a pivotal role in maintaining immunoregulation. Polymorphism in FOXO3a has been linked to inflammatory diseases such as chronic obstructive pulmonary disease (COPD), Rheumatoid Arthritis, and Crohn's disease suggesting that FOXO3a may be associated with asthma. Airway inflammation in asthma is characterized by activation of T helper type 2 (Th2) T cells and Foxo family members are reported to play critical roles in the suppression of T cell activation. Thus this study was undertaken to investigate an association between single nucleotide polymorphism of the FOXO3a (rs13217795, C>T transition) gene and asthma in Indian population. To our knowledge we are the first ones reporting an association between FOXO3a and asthma. Significant level of change has been seen in overall distribution of FOXO3a (rs13217795) genotypes in Healthy control group as compared to disease group although Healthy control group showed little increase in 'CC' genotype as compared to Patients of Asthma(54.46% *vs* 35.41%). Similarly, mutant type 'TT' genotype was present in low frequency in Asthma patients' group 12.50% and also in control group 09.82% (χ^2 = 7.643, P= 0.0219*). 'CT'genotype is higher in patients' group then control group (52.08% *vs* 35.71%)allele frequency was also significantly different (χ^2=5.544, P=0.0185*) whereas carriage rate was not significantly associated (χ^2 = 2.513, P= 0.1129ns).

1. INTRODUCTION:

Bronchial asthma is the most prevalent chronic immunological disorder in childhood period. It is characterized by airways inflammation and bronchial hyper-responsiveness where complex gene-environment interactions play a significant role in its pathogenesis. Asthma is a disorder whose primary cause can probably be linked to the disturbed immunoregulatory mechanisms at the lymphocyte level [1,3]. Inflammation in asthma occurs due to excessive infiltration of cells predominantly T lymphocytes, eosinophils, neutrophils, macrophages and mast cells along with elevated levels of cytokines and tumor necrosis factor-α.4 FOX (Forkhead box) proteins are a family of transcription factors that bind condensed chromatin during cell differentiation and play important roles in regulating the expression of genes involved in cell growth, proliferation, differentiation, and longevity [2]. FOXO3 is a member of the forkhead box class O (FOXO) subfamily6. The role of FOXO family members in inflammation is a complex role involving wide range of target cells and genes [1-3].

FOXO3 inhibits T cell proliferation, induces T cell apoptosis via upregulation of proapoptotic proteins such as Puma and Bim and it suppresses T cell activation preventing autoimmunity. Moreover, FOXO3 inhibits the capacity of dendritic cells to produce IL-6 so it can control the magnitude of T cell in immune responses.8 FOXO3 also promotes B cell apoptosis via upregulation of proapoptotic genes and antiproliferative genes. Forkhead (FOX) transcription factors play key roles in immunoregulation and homeostasis [4]. FOXO is the subfamily of FOX which comprises four members FOXO1, FOXO3a, FOXO4, and FOXO6. The FOXO genes are the mammalian homologs of *Caenorhabditis elegans* DAF-16 which regulate a number of pathways such as insulin signalling, apoptosis, cell cycle transition, DNA repair, oxidative stress resistance, and

[1]Corresponding Author can be contacted at: Centre for Biotechnology Studies, APS University, Rewa (M.P.)
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longevity [3,5]. Out of the four members of FOXO group only FOXO3a was found responsible for longevity in Chinese, Japanese, German, and Danish individuals [6]. FOXO3a is an integral component of protein kinase B/Akt pathway. Akt protein is responsible for cell proliferation in the presence of growth factors thereby suppressing the transcription of FOXO3a gene [7]. FOXO3a is phosphorylated, rendering it inactive, and is transported to the cytoplasm from nucleus via 14-3-3 chaperones. In case of inactivation of Akt, FOXO3a transcribes to produce p53, PTEN, Bim-1, FasL, GaDD45, and cyclin G2 promoting cell death or cell cycle arrest. Polymorphism in the FOXO3a gene leads to loss of control over the cell cycle leading to lymphoproliferation which results in formation of tumors and cancers such as prostate cancer and acut lymphoblastic leukemia [4-7].

FOXO3a had been reported to have redundant roles in suppressing inflammatory cytokine production by dendritic cells and initiation of TGFβ-1 dependent pathway in monocytes [8]. Through TGFβ-1 pathway, FOXO3a reduces production of proinflammatory cytokines including TNF-α, IL-4, and IL-13 and increases production of antiinflammatory cytokine IL-1. Rheumatoid Arthritis studies revealed that phosphorylation of FOXO3a gene took place in lymphocytes,monocytes, and macrophages implying FOXO3a's role in inflammatory cell activation. FOXO3a deficient mice models when triggered for cell proliferation exhibited lymphoproliferation, inflammation of airways, salivary glands, lungs, and a remarkable increase in activity of helper T cells. Survival of neutrophils, mast cells, and macrophages has also been associated with FOXO3a [9]. Polymorphism studies of FOXO3a have also proven association of FOXO3a and inflammatory diseases as chronic obstructive pulmonary disease, Rheumatoid Arthritis, Crohn's disease, and inflammatory bowel's disease [10]. Thus based on these studies we hypothesized that the hyperactivity of T cells, neutrophils, and mast cells, increased production of proinflammatory cytokines, and downregulation of anti-inflammatory cytokines in asthma patients may be linked to the polymorphism of FOXO3a gene [8-10].

2. MATERIALS AND METHODS:

2.1 Study population:

The study population consisted of 208 unrelated subjects comprising of 96 Asthma patients and 112 ethnically matched controls of central Indian population were included in this study. In this region Hindu, Muslim and some Sikh peoples are mainly living but most people's belong to Hindu religion in this region.

2.2 Anthropometric and Biochemical Measurements:

Body Mass Index (BMI) was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured in standing position midway between iliac crest and lower costal margin and hip circumference was measured at its maximum waist to hip ratio (WHR) was calculated using waist and hip circumferences. Systolic and diastolic blood pressures were measured twice in the right arm in sitting position after resting for at least 5 minute using a standard sphygmomanometer and the average of the two reading was used. Biochemical parameters related to type 2 diabetes were estimated for both cases and controls subjects. Measurement of Serum levels of Total cholesterol (TC), Triglycerides (TG), HbA1c, High density lipoprotein-cholesterol (HDL-C), Low density lipoprotein-cholesterol (LDL-C) and Urea were measured based on spectrophotometric method using automated clinical chemistry analyzer Cobas Integra 400 plus (Roche Diagnostics, Mannheim, Germany).

2.3 Blood collection and plasma/serum separation:

Venous blood samples were obtained from the subjects after 12 hours of overnight fasting in vacutainers with and without appropriate anti-coagulants. Immediately, plasma and serum from the respective vacutainers were separated by centrifuging the tubes at 1000 rpm for 10 min. at 4˚C

2.4 Method for DNA isolation:

Genomic DNA was extracted from whole blood by the modification of salting out procedure described by Miller and coworkers (Miller *et al*. 1988). Frozen blood sample was thawed at room temperature. 0.5 ml. of whole blood sample was suspended in 1.0 ml. of lysis buffer (0.32 M Sucrose, 1 mM Mgcl2, 12 mM Tris and 1% Triton-X-100) in a 1.5 ml. microcentrifuge tubes. Mixture was well frothed with the help of micro tip to allow proper lysis of pelleted nuclei. After digestion was complete, 100 µl. of saturated cold 5M Nacl was added and shaken vigorously for 15 sec. To the above mixture 0.2 ml. of deionized, autoclaved water and 0.4 ml. of phenol-chloroform (4:1 v/v) was added to remove most of the non nucleic acid organic molecules. Supernatant was discarded. The pellet was washed twice with 1 ml. of 70% alcohol. The pelleted DNA was rehydrated in 100-200 µl. of TE buffer pH 7.4 (10 mM Tris-HCL pH 7.4, 1mM EDTA, pH 8.0). DNA was allowed to dissolve overnight at 37˚C before quantization.

2.5 Polymorphism screening:

All the PCRs were carried out in a PTC 200 thermal cycler (MJ Research Inc. USA). PCR is a rapid, inexpensive and simple mean of producing relatively large copy number of DNA molecules from the small amounts of source DNA material, even when the source DNA is of relatively poor quality. Subsequently restriction enzyme digestion was performed by incubating the double stranded DNA with appropriate amount of restriction enzyme, in its respective buffer as recommended by the supplier and at optimal temperature for that specific enzyme. The PCR and restriction digestion conditions were optimized for specific locus of relevant segment of the gene to be studied. The PCR products as well as the digested products were separated on either agarose gel or polyacrylamide gel depending on their size. Gels were stained with ethidium bromide solution (0.5 µg/ml) and subsequently visualized and photographed under UV transilluminator.

2.6 Detection of FOXO3a (rs13217795) Single Nucleotide Polymorphism:
According to the SNP database, variant rs13217795 is located in intron 2 (Chr 6:108652895) of the FOXO3 gene.

*2.7 Primer sequence***:** The oligonucleotides sequences (primers) used were those described by NMEl Rifai.
Forward primer: 5'- 5′ CTC CTT GGT CAG TTT GGT G 3′-3'
Reverse Primer: 5' - ATG AGT GAA GAT GGA AGT AAG C -3'

2.8 PCR Mix:
The PCR was carried out in a final volume of 25 µl, containing 50-100 ng of genomic DNA(4-5 µl), 2.5 µl of 10X *Taq* polymerase buffer (10 mM Tris HCl pH 8.8, 50 mM KCl, 1.5 mM MgCl2, 0.01% gelatin, 0.005% Tween-20, 0.005% NP-40; final concentration 1X; Genetix Biotech Asia Pvt. Ltd.,India), 1 µl of 10 mM dNTPs (Banglore Genei, Bangalore, India), 1 µl of 25 pmol/µl of forward and reverse primers specific for and 1 µl of unit of 1U/ µl Red Taq DNA polymerase (Bangalore genei).

2.9 PCR Thermal Program:
After an initial denaturation of 5 min at 95˚C, the samples were subjected to 35 cycles at 95˚C for 1 min, at 58˚C for 45 s, and 72˚C for 45 s, with a final extension of 10 min at 72˚C in a thermal cycler. A 100bp ladder with amplified product has been run under 2.5 % agarose gel electrophoresis.

2.10 Restriction digestion:
PCR amplification of ***FOXO3a (*rs13217795)** Gene with specific primers gave 667bp undigested product which was digested with*PagI* enzyme (New England Biolabs, Boverly, MA) for 16 h at 37˚C. This reaction yielded one fragment of 667 bp indicating a homozygous wild genotype (CC), or two fragments of 391 and 275 bp indicating a homozygous mutant genotype (TT) while the presence of 677,391 and 275 bp products indicated heterozygous genotype (CT).The digestion products were then separated by electrophoresis on a 2.5% agarose gel. The results were documented by digital camera and further saved by gel documentation system.

3. RESULTS:

3.1 Anthropometric results:
The descriptive data and comparison of anthropometric and biochemical parameters of Asthma patients versus controls are presented in Table no.1. The age, sex, BMI, WHR were the parameters, revealed, Asthma patients were not different to healthy population. age group(P=0.5261ns), weight of women(P=0.1701ns) then men (P=0.5045ns) and BMI of Women (P=0.0896) then Men (P=0.1913ns) were not associated with Asthma. WHR was not significantly different between patient and healthy population. Thus, WHR in Women (P=0.2904ns) and Men (P=0.1912ns) were not found were not found association with Asthma**.**

Table -1. Comparative studies of anthropometric parameters

Characteristics	Cases (96)	Controls(112)	P-value
n(Men/Women)	96(54/42)	112(62/50)	
Age(years)	53.6±12.4	52.5±12.5	0.5261, ns
Height(m)	162.50±11.3	161.2±12.4	0.4333, ns
Weight (Kg)			
Women	61.5 ±4.7	60.6 ± 4.5	0.1701, ns
Men	68.4±5.6	67.8±7.1	0.5045,ns
BMI (kg/m^2)			
Women	23.6±3.1	22.7 ± 4.3	0.0896, ns
Men	24.6±4.7	23.8± 4.1	0.1913,ns
Waist circumference (cm)			
Women	92.5±6.2	93.6±6.7	0.2233,ns
Men	90.0±7.0	89.0±6.0	0.2685,ns
Hip (cm)			
Women	95.9±2.4	96.1±2.2	0.5315,ns
Men	90.8±4.3	91.2±1.5	0.3579,ns
WHR			
Women	0.97±0.05	0.98±0.08	0.2904,ns
Men	0.98±0.08	0.99±0.01	0.1912,ns

(*denotes level of significant change between case and control)

3.2 Life style factor results:
Life style factor like Rural/Urban, Alcoholic/Non-Alcoholic, Smoking/Non-Smoking and Tobacco chawing/Not-Chawing were selected for our population. We collected data from rural and urban life style. Three parameters Rural/Urban (P=0.8774), Alcoholic/Non-Alcoholic (P=0.6062) and Tobacco chawing/Not-Chawing (P=0.5993) were not associated with Asthma whereas factor Smoking/Non-Smoking (P=0.0004***) significantly associated to causing asthma **(See Table 2).**

Table 2. Comparative studies of Life Style

Characteristics	Cases (96)	Controls (112)	P-value
Rural/Urban	37/59	42/70	X^2 =0.02381 P=0.8774
Alcoholic/Non-Alcoholic	42/54	53/59	X^2 =0.2657 P=0.6062
Smoking/Non-Smoking	56/40	38/74	X^2 =12.43 P=0.0004***
Tobacco chawing/Not-Chawing	36/60	46/66	X^2 =0.2761 P=0.5993

3.2 Biochemical and clinical findings:

Biochemical test performed in the blood sample for following clinical parameters and the findings were tabulated. Statistical analysis was done by using student's t-test and p value suggests the level of significant were changed here. The descriptive data and comparison of biochemical parameters of Asthma patients versus healthy controls are presented in Table no. 4.3. As expected, the Asthma patients had markedly higher levels of Serum IgE (P<0.0001***) and Serum calcium (P<0.0001***) whereas level of Post-Prandial Glucose (mg/Dl), HbA1C (%), Blood Urea(mg/dL),HDL-C, LDL-C, TG, Systolic BP, Diastolic BP in Asthma patient was not significantly different to control group. Thus P-Value of Serum IgE (P<0.0001***) and Serum calcium (P<0.0001***) revealed association with Asthma**(See Table 3).**

Table 3. Comparative studies of Biochemical and clinical findings

Characteristics	Cases (96)	Controls(112)	P-value
Post-Prandial Glucose (mg/Dl)	117.7±12.4	118.4±11.6	0.6747,ns
HbA1C(%)	5.8±0.7	5.7±0.8	0.3424,ns
HDL-C(mmol/L)	108.8±12.2	109.3±11.6	0.7625,ns
LDL-C (mg/dL)	42.1±2.6	41.8±3.7	0.5063, ns
TG(mg/dL)	125.9±13.2	126.2±12.2	0.8650, ns
Systolic BP (mmHg)	125.4±8.1	124.8±5.7	0.5332, ns
Diastolic BP (mmHg)	83.1±5.8	82.5±6.2	0.4744, ns
Blood Urea(mg/dL)	18.1±1.7	17.8±1.8	0.2204,ns
Serum IgE (mg/dL)	606.4±287.7	107.8±85.8	P<0.0001***
Serum calcium (mmol/L)	2.3±0.4	2.8±0.3	P<0.0001***

(* denotes the level of significant change between case and control)

3.3 ELISA Analysis of IL-6 cytokine:

IL-6 (interleukin-6) plays an important role in adaptive immune responses little is known about their role(s) in the thrombo-inflammatory responses associated with Ang II. Concentration of IL-6level in Asthma patient (case) and healthy population (control) is depicted in table no.-4.3, is showing decreased level during Asthmaand it was statistically significant associated as P<0.0001*** with Asthma**.** Graph no. 4.3 is showing ELISA of IL-6 result. This is kit-based ELISA result reveals elevated IL-6 level in essential hypertension. In figure no. 4.3, two columns ELISA analysis having four standards A, B, C, D containing 25, 50, 100, 200 Pg/ml concentration respectively read absorbance at 450 nm. G for Patient (Case) and H for Healthy (control) showing differences in IL-6 level as 1.14 mIU/L and 1.67 mIU/L respectively.

Table .4. Comparison of Biochemical Factor between Autoimmune Thyroid Disease (AITD) Cases and Healthy Controls:

Characteristics	Cases (96)	Controls(112)	P-value
IL-6 Level in serum (mIU/L)	1.14±0.40	1.67±0.60	P<0.0001***

(*Denotes level of significant change between malarial cases and healthy controls.)

3.4 Detection of Genetic Polymorphism in FOXO3a (rs13217795) Gene:

PCR amplification of **FOXO3a(rs13217795)** Genewith specific primers gave 538-bp undigested productwhich was digested with *PagI* enzyme (New England Biolabs, Boverly, MA) for 16 h at 37˚C. The wild-type genotype (CC) wasnot digestedand give 667bp whereas the mutated homozygous genotype (TT) was cut as 391and 275bp. The heterozygous genotype (TC) was represented as fragments of 667, 391, and 275bp of DNA fragments in the gel.

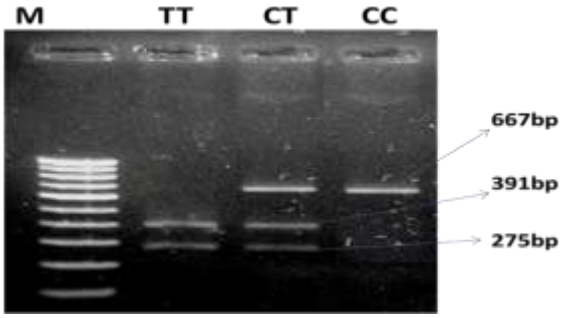


Fig.-1: Representative gel picture of FOXO3a(rs13217795) polymorphism. Lane M represents 50 bp molecular marker, Lane CC Wild type genotype, Lane CT heterozygous genotype and Lane TT variant genotype.

The distribution of the polymorphisms of FOXO3a (rs13217795) was consistent with Hardy- Weinberg equilibrium (HWE) in healthy controls. The observed genotype frequencies, allele frequencies and carriage rates for FOXO3a (rs13217795) polymorphism are depicted in table 4.4 and table 4.5 and Graph 4.1, 4.2, 4.3. Significant level of change has been seen in overall distribution of FOXO3a (rs13217795) genotypes in Healthy control group as compared to disease group although Healthy control group showed little increase in 'CC' genotype as compared to Patients of Asthma (54.46% *vs* 35.41%). Similarly, mutant type 'TT' genotype was present in low frequency in Asthma patients' group 12.50% and also in control group 09.82% (χ^2 = 7.643, P= 0.0219*). 'CT' genotype is higher in patients' group then control group (52.08% *vs* 35.71%) allele frequency was also significantly different (χ^2=5.544, P=0.0185*) whereas carriage rate was not significantly associated (χ^2 = 2.513, P= 0.1129ns). An odds ratio of CC genotype was calculated as0.4585 which indicates little protective effect of CC genotype from disease and indicate significantly differences between patient and healthy group (P=0.0079**). An odds ratio of TC and TT genotype was not indicating as protective effect. Significant difference in Allele frequency between Asthma patients' group and healthy control group indicate association with Asthma (P=0.0211*) but not show protective effect whereas carriage rate was not significantly different. Overall allele 'C' was found little lower frequency in disease group as compared to HC group (61.45% Vs 72.32%) whereas allele '*T*' was present in little high frequency in the disease group was significantly different. Carriage rate of allele 'T' was slightly high in Asthma group as compared to healthy control (42.46% Vs 33.55%) whereas carriage rate of allele '*C*' was nominal different in both control and disease group but no significant level of change has been seen. The pattern of genotype distribution, allele frequency and carriage rate in disease and control group suggests FOXO3a (rs13217795) polymorphism is significantly associated with Asthma in our population.

Table 6. Frequency distribution and association of Genotype, allele frequency and carriage rate of FOXO3a (rs13217795) gene polymorphism in population of Vindhyan region using Chi Square Test

***FOXO3a* GENE**	**CASE N= 96**		**CONTROL N=112**		**CHI SQUARE VALUE χ^2 (P Value)**
	N	**%**	**N**	**%**	
Genotype					
CC	34	35.41	61	54.46	
CT	50	52.08	40	35.71	
TT	12	12.50	11	09.82	7.643(0.0219*)
Allele					
C	118	61.45	162	72.32	
T	74	38.54	62	27.67	5.544 (0.0185*)
Carriage Rate					
C	84	57.53	101	66.44	
T	62	42.46	51	33.55	2.513 (0.1129ns)

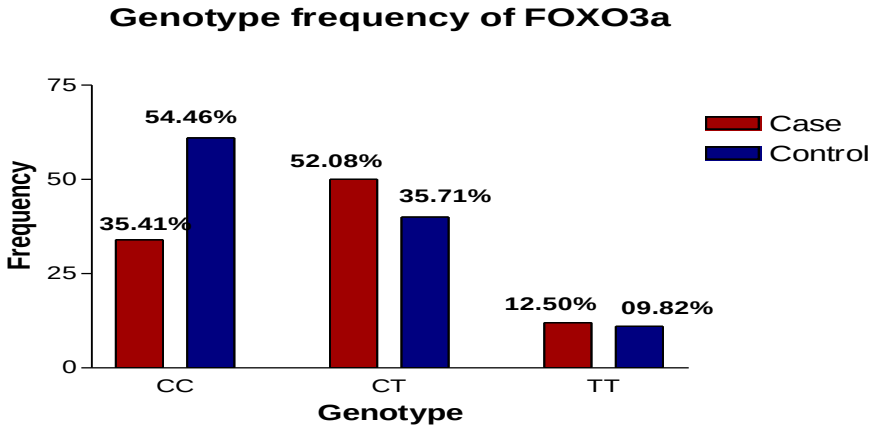
(- denotes the level of significant association between case and control.);*
(N – Number of individuals in study group.)
(% - Genotype allele frequency and carriage rate expressed in percentage.)

Table .7. Fisher Exact Test values of FOXO3a (rs13217795) gene polymorphism

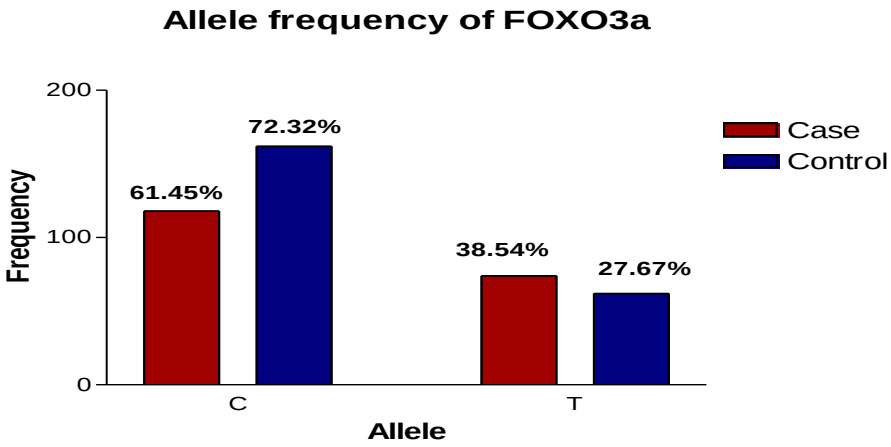
***FOXO3a* GENE**	**CASE N= 96**		**CONTROL N=112**		**P Value**	**Odds Ratio (95% confidence interval)**
	N	**%**	**N**	**%**		
Genotype						
CC	34	35.41	61	54.46	0.0079**	0.4585 (0.2619 to 0.8025)
CT	50	52.08	40	35.71	0.0245*	1.957 (1.121 to 3.414)
TT	12	12.50	11	09.82	0.6584ns	1.312 (0.5506 to 3.125)
Allele						
C	118	61.45	162	72.32		0.6103 (0.4040 to 0.9220)
T	74	38.54	62	27.67	0.0211*	1.639 (1.085 to 2.475)

Carriage Rate						
C	84	57.53	101	66.44		0.6841 (0.4275 to 1.095)
T	62	42.46	51	33.55	0.1218ns	1.462 (0.9133 to 2.339)

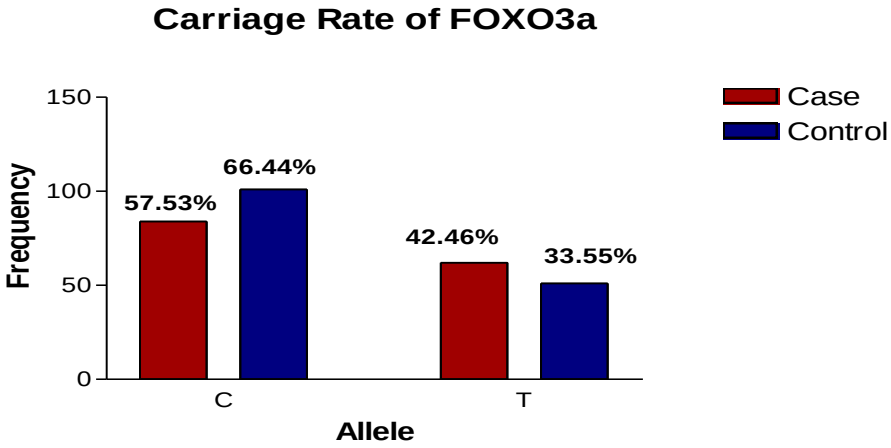
(- denotes the level of significant association between case and control.)*
(N – Number of individuals in study group.)
(% - Genotype allele frequency and carriage rate expressed in percentage.)



Graph No.-1:Genotype Frequency of *FOXO3a*gene.



Graph No.-2:Allele Frequency of *FOXO3a* gene.



Graph No.-3: Carriage rate of *FOXO3a*gene.

4. DISCUSSION:

The relationship between asthma, smoking, and anthropometric measurements such as body mass index (BMI), waist circumference (WC), hip circumference (HC), and waist-to-hip ratio (WHR) among individuals residing in the United States. They classified the participants into four groups based on self-reported smoking and asthma status: non-smokers with no asthma, asthma alone, smokers only, and smokers with asthma. The outcomes of interest were BMI, WC, HC, and WHR scores in the latter three groups compared to the non-smokers with no asthma group [12]. Linear regression analysis showed that those with asthma alone and smokers with asthma were significantly more likely to have a BMI, WC, or HC score of 1 or higher than people without asthma and smokers only. A higher score on the anthropometric

parameters was substantially related to participants who had only asthma and those who had both asthma and smoking [11-13].

Our descriptive data from comparative study of anthropometric and biochemical parameters of Asthma patients versus controls are presented. The age, sex, BMI, WHR were the parameters. As expected, the Asthma patients had markedly higher levels of high age group (P<0.0001***), weight of women (P<0.0001***) then men (P=0.5045ns) and BMI of Women (P<0.0001***) then Men (P=0.1913ns). The parameter Waist circumference (cm) and Hip (cm) determine the WHR. Our statistical data from Waist circumference (cm) and Hip (cm) was not significant different between Asthma patients' group and healthy control (HC) group. WHR was not significantly different between patient and healthy population. Thus, WHR in Women (P=0.2904ns) and Men (P=0.1912ns) were not found were not found association with Asthma.

Asthma is the most common childhood illness and disproportionately affects low-income, minority children who live in urban areas. A range of risk factors are associated with asthma morbidity and mortality, such as treatment non-adherence, exposure to environmental triggers, low-income households, exposure to chronic stress, child psychological problems, parental stress, family functioning, obesity, physical inactivity, and unhealthy diets [8,16]. These risk factors often have complex interactions and inter-relationships. Comprehensive studies that explore the inter-relationships of these factors in accounting for asthma morbidity and mortality are needed and would help to inform clinical intervention. Lifestyle interventions also hold great promise for asthma sufferers as they are accessible, low cost and have minimal side-effects, thus making adherence more likely [15,17]. This review explores lifestyle interventions that have been tested in asthma, including improving nutrition, increasing physical activity and introduction of relaxation therapies such as yoga and massage therapy. Available evidence suggests a protective effect of increasing fruit, vegetable and wholegrain intake and increasing physical activity levels in asthma. Weight loss is recommended for obese asthmatic patients, as just 5–10% weight loss has been found to improve quality of life and asthma control in most obese asthmatic patients [14-17]. Our statistical data from Life style factor like Rural/Urban, Alcoholic/Non-Alcoholic, Smoking/Non-Smoking and Tobacco chawing/Not-Chawing were indicate association of asthma. We collected data from rural and urban life style. Three parameters Rural/Urban (P=0.8774), Alcoholic/Non-Alcoholic (P=0.6062) and Tobacco chawing/Not-Chawing (P=0.5993) were not associated with Asthma whereas factor Smoking/Non-Smoking (P=0.0004***) significantly associated to causing asthma.

Biomarker analysis was developed to identify inflammation in the asthmatic airway. It has led to a renewed interest in biochemical abnormalities in the asthmatic airway. The biochemical determinants of asthma heterogeneity are many [19]. Examples include decreased activity of superoxide dismutases; increased activity of eosinophil peroxidase, S-nitrosoglutathione reductase, and arginases; decreased airway pH; and increased levels of asymmetric dimethyl arginine. New discoveries suggest that biomarkers such as exhaled nitric oxide reflect complex airway biochemistry [18]. This biochemistry can be informative and therapeutically relevant. Improved understanding of airway biochemistry will lead to new tests to identify biochemically unique subpopulations of patients with asthma It will also likely lead to new, targeted treatments for these specific asthma subpopulations [21]. Serum trace elements: Zinc and copper, malondialdehyde, vitamin C, vitamin E, immunoglobulin A and immunoglobulin E were estimated using standard methods. There serum levels of copper, IgE, IgA and MDA were significantly increased in asthmatic patients when compared to control ($p< 0.05$). These observations probably indicated that asthmatic patients could be prone to the oxidative stress [18-21].

Our result from Biochemical test performed in the blood sample for following clinical parameters and the findings were tabulated. Statistical analysis was done by using student's t-test and p value suggests the level of significant were changed here. The descriptive data and comparison of biochemical parameters of Asthma patients versus healthy controls are presented in Table no. 4.3. As expected, the Asthma patients had markedly higher levels of Serum IgE (P<0.0001***) and Serum calcium (P<0.0001***) whereas level of Post-Prandial Glucose (mg/Dl), HbA1C (%), Blood Urea(mg/dL), HDL-C, LDL-C, TG, Systolic BP, Diastolic BP in Asthma patient was not significantly different to control group. Thus P-Value of Serum IgE (P<0.0001***) and Serum calcium (P<0.0001***) revealed association with Asthma.

Bronchial asthma (BA) is a complex disease characterised by persistent inflammation. Exhaled nitric oxide (FeNO) and blood eosinophil count (b-Eos) are biomarkers for type 2 endotype of BA. Serum levels of IL-5, IL-6, IL-8, IL-13 and IL-17A (ELISA) were measured in 30 healthy controls (HC) and 80 adult BA patients. All interleukins and total IgE were significantly higher in patients with BA as compared with HC. IL-5 levels were highest in Group 2 ($p < 0.05$). IL-6, IL-13 and IL-17A levels were elevated in Groups 2, 3 and 4 as compared with HC ($p < 0.05$). Higher IL-8 levels were associated with a pattern of current smokers. Highest IL-17A levels were found in type 2 high groups with frequent exacerbations, mostly uncontrolled and severe BA. We have found a distinct pattern for each group based on demographic, clinical, functional, immunological and inflammatory characteristics. FeNO and b-Eos are useful in the identification of severe type 2 BA subgroups with frequent exacerbations. IL-5, IL-6, IL-13 and IL-17A are involved in the persistent type 2 immune response in moderate and severe BA [21-23]. Our cytokine investigation of IL-6 (interleukin-6) plays an important role in adaptive immune responses little is known about their role(s) in the thrombo-inflammatory responses associated with Ang II. Concentration of IL-6level in essential Asthma patient (case) and healthy population (control) is depicted in table no.-4.3, is showing decreased level during essential Asthma and it was statistically significant associated as P<0.0001*** with Asthma. This is kit-based ELISA result reveals elevated IL-6 level in Asthma. In figure no. 4.3, two columns ELISA analysis having four standards A, B, C, D containing 25, 50, 100, 200 Pg/ml concentration respectively read absorbance at 450 nm.

G for Patient (Case) and H for Healthy (control) showing differences in IL-6 level as 1.14 mIU/L and 1.67 mIU/L respectively.

Asthma is a chronic inflammatory disorder delineated by a heightened immunological response due to environmental or genetic factors. Single nucleotide polymorphism studies have shown that FOXO3a plays a pivotal role in maintaining immunoregulation. Polymorphism in FOXO3a has been linked to inflammatory diseases such as chronic obstructive pulmonary disease (COPD), Rheumatoid Arthritis, and Crohn's disease suggesting that FOXO3a may be associated with asthma. Airway inflammation in asthma is characterized by activation of T helper type 2 (Th2) T cells and Foxo family members are reported to play critical roles in the suppression of T cell activation [22]. FOXO3a proteins play multiple crucial roles in immune response. FOXO3 inhibits T cell proliferation, induces T cell apoptosis via upregulation of proapoptotic proteins and it suppresses T cell activation preventing autoimmunity. The role of FOXO3a gene in the pathogenesis of bronchial asthma has been studied in few ethnic groups and revealed its implication in asthma pathogenesis. The highest frequency was for the heterozygous type CT in both cases and controls groups. The genotype frequencies of mutant type TT for cases and controls were 12 % and 16% respectively, and the T allele frequencies were 37.2% in cases and 46.7% in the control group while CC genotype was present in 37.3% of asthmatic patients and 22.6% in the controls and the C allele was detected in 62.8% and 53.3% for cases and controls respectively [21-23].

Our statistical data from the distribution of the polymorphisms of FOXO3a (rs13217795) was consistent with Hardy-Weinberg equilibrium (HWE) in healthy controls. The observed genotype frequencies, allele frequencies and carriage rates for FOXO3a (rs13217795) polymorphism are investigated in our population. Significant level of change has been seen in overall distribution of FOXO3a (rs13217795) genotypes in Healthy control group as compared to disease group although Healthy control group showed little increase in 'CC' genotype as compared to Patients of Asthma (54.46% *vs* 35.41%). Similarly, mutant type 'TT' genotype was present in low frequency in Asthma patients' group 12.50% and also in control group 09.82% (χ^2 = 7.643, P= 0.0219*). 'CT'genotype is higher in patients' group then control group (52.08% *vs* 35.71%) allele frequency was also significantly different (χ^2=5.544, P=0.0185*) whereas carriage rate was not significantly associated (χ^2 = 2.513, P= 0.1129ns). An odds ratio of CC genotype was calculated as 0.4585 which indicates little protective effect of CC genotype from disease and indicate significantly differences between patient and healthy group (P=0.0079**). An odds ratio of TC and TT genotype was not indicating as protective effect. Significant difference in Allele frequency between Asthma patients' group and healthy control group indicate association with Asthma (P=0.0211*) but not show protective effect whereas carriage rate was not significantly different. Overall allele 'C' was found little lower frequency in disease group as compared to HC group (61.45% Vs 72.32%) whereas allele '*T*' was present in little high frequency in the disease group was significantly different. Carriage rate of allele 'T' was slightly high in Asthma group as compared to healthy control (42.46% Vs 33.55%) whereas carriage rate of allele '*C*' was nominal different in both control and disease group but no significant level of change has been seen. The pattern of genotype distribution, allele frequency and carriage rate in disease and control group suggests FOXO3a (rs13217795) polymorphism is significantly associated with Asthma in our population [24]. The asthma is a chronic inflammatory process that affects the airways of the lung. It has a greater impact on public health. It is a polygenic disease, many factors play a role implicated in triggering it's that starts like genetic, environmental, and geographic factors [25]. Genetic studies have focused on the fork head box O3a (FOXO3a) gene, mainly polymorphism at the specific sites which is thought may contribute to exacerbating the attack and complications of asthma.

5. CONCLUSION

The study was designed as a case-control and involved 138 of asthmatic patients and 120 seemingly healthy people used as a control group. Genotyping of all subjects was done for FOXO3a(rs13217795; C/T) analysis by PCR-RFLP technique [15,26]. Multinominal regression was used to calculate Odds ratios (ORs) and 95%confidence intervals (CIs). Analysis of the results that obtained from this work a considerable modification in forms of rs13217795; C/T polymorphism between asthmatic and healthy individuals. elevation in the risk of asthmatic patient was seen in T allele of FOXO3a gene (odds ratio of 2.0313, 95% CI: 1.5493 -2.6631, P<0.0001). Conclusions: We concluded that patients who carry the T/T allele are at high risk for developing illness and complications compared with 6075other forms which included C/T and C/C alleles, respectively (P < 0.0001), and patients with the C/T allele showed increased susceptibility with border-line significance. They revealed that FOXO3a (rs13217795; C/T) polymorphism has a significant role in the development and progression of asthma [24-27].

REFERENCES:

1. Lunetta KL, Murabito JM. Genetics of human longevity and healthy aging. In: Newman AB, Cauley JA, editors. The Epidemiology of Aging. New York: Springer; 2012. p. 215–35.
2. Ziv E, Hu D. Genetic variation in insulin/IGF-1 signaling pathways and longevity. *Ageing Res Rev* 2011; 10: 201–4.
3. Bonafe M, Olivieri F. Genetic polymorphism in long-lived people: cues for the presence of an insulin/IGF-pathway-dependent network affecting human longevity. *Mol Cell Endocrinol* 2009; 299: 118–23.
4. Willcox BJ, Donlon TA, He Q, Chen R, Grove JS, *et al*. *FOXO3A* genotype is strongly associated with human longevity. *Proc Natl Acad Sci U S A* 2008; 105: 13987–92.
5. Anselmi CV, Malovini A, Roncarati R, Novelli V, Villa F, *et al*. Association of the *FOXO3A* locus with extreme longevity in a southern Italian centenarian study.. *Rejuvenation Res* 2009; 12: 95–104.

6. Flachsbart F, Caliebe A, Kleindorp R, Blanche H, von Eller-Eberstein H, *et al.* Association of *FOXO3A* variation with human longevity confirmed in German centenarians. *Proc Natl Acad Sci U S A* 2009; 106: 2700–5.
7. Li Y, Wang WJ, Cao H, Lu J, Wu C, *et al.* Genetic association of *FOXO1A* and *FOXO3A* with longevity trait in Han Chinese populations. *Hum Mol Genet* 2009; 18: 4897–904.
8. Kleindorp R, Flachsbart F, Puca AA, Malovini A, Schreiber S, *et al.* Candidate gene study of *FOXO1*, *FOXO4*, and *FOXO6* reveals no association with human longevity in Germans. *Aging Cell* 2011; 10: 622–8.
9. Tzivion G, Dobson M, Ramakrishnan G. FoxO transcription factors; Regulation by AKT and 14–3–3 proteins. *Biochim Biophys Acta* 2011; 1813: 1938–45.
10. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics* 1994; 50:1088–101.
11. Pawlikowska L, Hu D, Huntsman S, Sung A, Chu C, *et al*. Study of Osteoporotic Fractures. Association of common genetic variation in the insulin/IGF1 signaling pathway with human longevity. *Aging Cell* 2009; 8: 460–72.
12. Li XJ, Zhang ZY, Qin J, Zhang N, Rong MH, *et al*. Correlation between *FOXO3A* gene polymorphisms and human longevity in Bama County, Guangxi Province. *J Environ Health* 2010; 1: 54–6.
13. Chung WH, Dao RL, Chen LK, Hung SI. The role of genetic variants in human longevity. *Ageing Res Rev* 2010; 9: S67–78.
14. Kuningas M, Magi R, Westendorp RG, Slagboom PE, Remm M, *et al.* Haplotypes in the human *Foxo1a* and *Foxo3a* genes; impact on disease and mortality at old age. *Eur J Hum Genet* 2007; 15: 294–301.
15. Zeng Y, Cheng L, Chen H, Cao H, Hauser ER, *et al*. Effects of *FOXO* genotypes on longevity: a biodemographic analysis. *J Gerontol A Biol Sci Med Sci* 2010; 65: 1285–99.
16. Capri M, Salvioli S, Sevini F, Valensin S, Celani L, *et al*. The genetics of human longevity. *Ann N Y Acad Sci* 2006; 1067: 252–63.
17. Herskind AM, McGue M, Holm NV, Sorensen TI, Harvald B, *et al*. The heritability of human longevity: a population-based study of 2872 Danish twin pairs born 1870–1900. *Hum Genet* 1996; 97: 319–23.
18. Skytthe A, Pedersen NL, Kaprio J, Stazi MA, Hjelmborg JV, *et al*. Longevity studies in Genom EU twin. *Twin Res* 2003; 6: 448–54.
19. Manolio TA, Brooks LD, Collins FS. A HapMap harvest of insights into the genetics of common disease. *J Clin Invest* 2008; 118: 1590–605.
20. Nebel A, Kleindorp R, Caliebe A, Nothnagel M, Blanche H, *et al*. A genome-wide association study confirms APOE as the major gene influencing survival in long-lived individuals. *Mech Ageing Dev* 2011; 132: 324–30.
21. Christensen K, Johnson TE, Vaupel JW. The quest for genetic determinants of human longevity: challenges and insights. *Nat Rev Genet* 2006; 7: 436–48.
22. Banasik K, Ribel-Madsen R, Gjesing AP, Wegner L, Andersson A, *et al*. The *FOXO3A* rs2802292 G-allele associates with improved peripheral and hepatic insulin sensitivity and increased skeletal muscle-*FOXO3A* mRNA expression in twins. *J Clin Endocrinol Metab* 2011; 96: E119–24.
23. Franceschi C, Motta L, Valensin S, Rapisarda R, Franzone A, *et al*. Do men and women follow different trajectories to reach extreme longevity? Italian Multicenter Study on Centenarians. *Aging (Milan)* 2000; 12: 77–84.
24. Corbo RM, Pinto A, Scacchi R. Gender-specific association between FSHR and PPARG common variants and human longevity. *Rejuvenation Res* 2013; 16: 21–7.
25. Barbieri M, Bonafe M, Rizzo MR, Ragno E, Olivieri F, *et al.* Gender specific association of genetic variation in peroxisome proliferator-activated receptor (PPAR) gamma-2 with longevity. *Exp Gerontol* 2004; 39: 1095–100.
26. Bonafe M, Olivieri F, Cavallone L, Giovagnetti S, Mayegiani F, *et al.* A gender-dependent genetic predisposition to produce high levels of IL-6 is detrimental for longevity. *Eur J Immunol* 2001; 31: 2357–61.
27. Tan Q, Yashin AI, Bladbjerg EM, de Maat MP, Andersen-Ranberg K, *et al*. Variations of cardiovascular disease associated genes exhibit sex-dependent influence on human longevity. *Exp Gerontol* 2001; 36: 1303–15.