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ORIGINAL ARTICLE

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IL-10(-592A/C) gene variant a predictor of postoperative atrial fibrillation in the north Indian population

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Abstract

Background: There is an accumulating body of evidence indicating a strong association between inflammation and the pathogenesis of atrial fibrillation (AF) in different ethnicities across the globe. AF increases the risk of stroke and heart failure. Despite various researches on *IL-10* response, there is limited clinical evidence present, which demonstrate a role of these immunity regulators in AF. Therefore, this study was designed to decipher the role of *IL-10(-592A/C)* polymorphism in the development of postoperative AF (post-OP AF).

Method: The study was designed for north Indian patients. The study included 90 patients with AF and 126 controls in sinus rhythm undergoing surgery at Department of Cardiovascular and thoracic surgery, SGPGIMS, Lucknow, India. DNA samples were genotyped for common single nucleotide polymorphism (SNP) in gene *IL-10(-592A/C)*. The PCR-based RFLP technique was used to assess the genotype frequencies. The multivariable logistic regression analysis was performed to study the association of other risk factors with AF.

Results: The distribution of *IL-10(-592A/C)* genotypes (CC, AC, and AA) was found to be 48.41%, 47.61%, and 3.98% in controls and 41.11%, 45.55%, and 13.34% in cases, respectively ($P = .0385$). The frequency of allele A in cases was significantly higher than the control group (36.11% vs 27.77%, $P = .0654$). Compared with CC, AA genotype had increased risk of AF in both unadjusted and adjusted analyses.

Conclusions: This study suggests that *IL-10(-592A/C)* polymorphism may have significant association with post-OP AF development in north Indian patients.

KEYWORDS
atrial fibrillation, cardiac surgery, genotype frequency, *IL-10(-592A/C)*, RFLP

1 | INTRODUCTION

Atrial fibrillation is a common heart rhythm disorder associated with mortality of life and is associated with the elevated socioeconomic burden across the globe. The severity of this disorder found to be enhanced in patients with myocardial infarction and stroke. The prevalence is estimated to be ≥1%, in adult population all over the world.[1,2] In case of Indian population, the prevalence of AF is still

not investigated in the general population; few studies suggested that rheumatic heart disease patients have a higher risk of AF.[3] The Previous researcher has demonstrated AF as a multifactorial disorder that is regulated by genetic as well as nongenetic factors.[4] Although several risk factors for post-OP AF have been elucidated in few studies, but the mechanism of post-OP AF development still need to be elucidated.[5]

A previous study in bypass surgery suggested that inflammation participated in the pathogenesis of post-OP AF.[5] *IL-10* is known as a major anti-inflammatory cytokine that plays an important role in the regulation of the immune system. It deactivates the inflammatory response mediated by macrophages and lymphocytes and inhibits the production of pro-inflammatory cytokines.[6–10] An increased production of *IL-10* might thus result in better control of inflammatory responses induced by chronic vessel damage and reduce the risk of atherogenic complications.[11] The precise mechanism by which genetic variants of *IL-10* modulate atrial electrophysiological properties or the structural background conferring susceptibility to AF still remains unidentified.[12] Therefore, this study was designed to decipher the role of genetic variant of *IL-10* in atrial fibrillation in Indian population. The present case-control single nucleotide polymorphism study is carried out in selected gene *IL-10-592* (A-C, rs1800872) located on chromosome 1 (1q31-1q32) to evaluate its association with atrial fibrillation in north Indian population for the first time. The SNP is polymorphic with minor allele frequencies of ≤33.4%, and the studied SNP followed HWE. The study has implications in the development of diagnostics to prevent severity associated with postoperative inflammation in patients undergoing cardiac surgery.

2 | METHODS

2.1 | Study population

The cases comprised of 90 post-OP AF patients admitted to Department of Cardio Vascular and Thoracic Surgery, SGPGIMS hospital between October 2015 and March 2016 and who were diagnosed with AF via serial electrocardiogram (ECG). Postoperatively, heart rate and rhythm were continuously monitored until 7 days. In this study, post-OP AF was defined as the characteristic arrhythmia lasting for ≥5 minutes and confirmed by ECG. ECG collected till 7 days to check whether patient has paroxysmal/permanent AF. The controls comprised of 126 patients with sinus rhythm. The selected patients in both groups underwent following coronary artery bypass grafting (CABG) or Valvular heart disease (VHD) surgery. Exclusion criteria were patients with history of AF including lone AF, or other significant supraventricular or ventricular arrhythmias, heart block, mitral stenosis, congenital heart defect, and other infections, or were taken antiarrhythmic drugs. The variables such as thyroid, alcohol, smoking, diabetes, hypertension, ACE inhibitors, beta-blockers, blood pressure, and pulse rate were also included in the study analysis as shown in (Table 1). The study protocol was approved by the Ethics Committee of SGPGIMS, Lucknow. Written informed consent was obtained from each participant.

TABLE 1 Demographic and clinical characteristics

Variable	Cases	Controls	*P*-value
Sex (male/female)	51/39	102/24	.0001
Age	44.3 ± 14	52.55 ± 14.33	.027
Diabetes	21.11%	42.5%	.002
Smoking	9%	13.33%	.812
Tobacco	7%	5%	.153
Hypertension	52.22%	73.55%	.0001
Sleep apnea	14.44%	17.5%	.817
Thyroid	5.6%	2%	.363
LVD	36.67%	37%	.909
LVEF	42.7	46.34	-
Family history of CAD	3.33%	4.2%	.529
Beta-blocker	45.5%	50%	.452
Angiotensin	44%	50%	.395
CABG	40%	69%	-
VHD	60%	31%	-
Systolic BP	116 ± 13.4	125 ± 17.62	-
Diastolic BP	74.5 ± 6.5	77.23 ± 9.42	-
Pulse rate	87 ± 7.13	82 ± 8.01	-
Drain	828.65	913.24	-
Ventilation time	19.35	18	-
ICU time	36.55	51.6	-
Ionotrope			
NOR	24.43	25	-
DOB	32.8	28.4	
ADR	29.43	31.54	
NTG	7.71	8	
Blood unit			
PRBC	2.25	2	-
PLT	5.33	2.5	
FFP	5.83	3	
Angina	10%	35%	-
MI	**5%**	**25%**	**-**

ADR, adrenaline; BP, blood pressure; CABG, coronary artery bypass graft; CAD, coronary artery disease; DOB, dobutamine; FFP, fresh frozen plasma; HT, hypertension; LVD, left ventricular dysfunction; LVEF, left ventricular ejection fraction; MI, myocardial infarction; NOR, noradrenaline; NTG, nitroglycerine; PLT, platelets; PRBC, packed red blood cells; VHD, valvular heart disease; Bold values indicate $p \leq 5\%$ significant.

2.2 | Molecular analysis

Genomic DNA extraction was performed from peripheral blood leukocytes using the phenol–chloroform method. The genotyping of *IL-10(-592A/C)* was performed through PCR–RFLP analysis. The PCR was conducted in a final volume of 30 μL using primers, 5′-AACTTCTT-CCACCCCATCTTT-3′ (sense) and 5′-ATCCTCAAA-

GTTCCCAAGCAG-3′ (antisense) at an annealing temperature of 55.5°C. The 16 μL of the reaction Greentaq Lucigen master mix, 2 μL of each primer, 8 μL nuclease-free water, and 200 ng genomic DNA were used for amplification. Cycling conditions included an initial denaturation at 94°C for 5 minutes followed by 38 cycles with a fast denaturation at 94°C for 40 seconds, an annealing step at 55.5°C for 38 seconds, and an extension step at 72°C for 40 seconds, with a final incubation at 72°C of 5 minutes. The amplification reaction was followed by a digestion with the reaction enzyme, *RsaI* (NEB), at 37°C for 2-3 hours and electrophoresis on 2.0% agarose gel. The polymerase reaction product was 465 base pairs in size. Amplification product was cut with *RsaI* to produce fragments resulted in CC genotype at 465 bp, AA at position 405 and 60 bp and AC at position of 465, 405, and 60 bp in electrophoresis.

2.3 | Statistical analysis

Statistical analysis was performed using the SPSS (Statistical Package for Social Sciences, Chicago, USA) software for Windows (Version 15.0). The statistical power of the study (>80%) was calculated with regard to the study type (case-control), prevalence of AF, and the frequency of the minor allele in the control population at the level of significance 5%. The Chi-square test was used to test the deviation of genotype distribution from Hardy-Weinberg equilibrium and the differences of the frequency of *IL-10(-592A/C)*. The association between the risk factors and AF was assessed using logistic regression analysis. Odds ratio (OR) with 95% confidence interval (CI) was determined. The threshold for statistical significance was a *P*-value of 5%.

3 | RESULTS

3.1 | Selection of the study subject

The total subjects selected in the study were 216. The subjects were divided into cases and controls according to the data obtained after the cardiac surgery. Therefore, 90 were enrolled as cases and 126 as controls. The subjects were enrolled in the Department of Cardiovascular and thoracic surgery at Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow. The subject's details were obtained from their history. A standardized questionnaire was prepared to record the data of subjects such as age, sex, surgery, rhythm, cardiac, and noncardiac comorbidities (Table 1). The presence of post-OP AF was determined by serial electrocardiogram till 7 days. The AF exist mainly on 2nd-4th days was therefore paroxysmal AF. The study was approved by the Ethics Committee of SGPGIMS, and written informed consent was obtained from all participants.

3.2 | Characteristics of the study subjects:

The demographic and clinical characteristics of the study population are shown in the table with age ($P = .027$) and sex (0.0001) matched (Table 1). The clinical characteristics of all participants shown no statistically significant differences between the two groups (cases vs controls) with regard to prevalence of hypertension (52.22 vs 73.55%, $P = .0001$), smoking (9 vs 13.33%, $P = .812$), sleep apnea (14.44 vs 17.5%, $P = .817$), tobacco (7 vs 5%, $P = .153$), thyroid (5.6 vs 2%, $P = .363$), LVD (36.67% vs 37%, $P = .909$), LVEF (42.7 vs 46.34), family history of CAD (3.33% vs 4.2%, $P = .529$), beta-blocker (45.5% vs 50%, $P = .452$), angiotensin (44% vs 50%, $P = .395$), CABG (40% vs 69%), VHD (60% vs 31%), systolic BP (116 ± 13.4 vs 125 ± 17.62), diastolic BP (74.5 ± 6.5 vs 77.23 ± 9.42), pulse rate (87 ± 7.13 vs 82 ± 8.01), drain (828.65 vs 913.24), ICU time (36.55 vs 51.6), ventilation time (19.35 vs 18), ionotropes (nor: 24.43 vs 25, dob:32.8 vs 28.4, adr 29.43 vs 31.54, ntg:7.71 vs 8) blood unit (PRBC:2.25 vs 2, PLT:5.33 vs 2.5, FFP 5.83 vs 3), angina (10% vs 35%), and MI (5% vs 25%). The significant observations were seen in cases of diabetes ($P = .002$) and age ($P = .027$) when compared with controls. However, compared with controls, AF patients were younger than the time of extubation was more in AF patients than controls in sinus rhythm (19.35 vs 18 hours).

3.3 | Distributions of *IL-10(-592A/C)* genotypes and allele frequencies:

PCR product when digested with *RsaI* yields 405- and 60-bp fragments when A is at position -592. According to electrophoresis, the fragments were 405 + 60 bp (AA genotype), 465 bp (CC genotype) and 465 + 405 + 60 bp (AC genotype), respectively. The distribution of *IL-10* genotypes investigated in both the groups significantly deviated from the Hardy-Weinberg equilibrium shown in Table. The distribution of the *IL-10(-592A/C)* genotypes (AA, AC, and CC) was 3.98%, 47.61% & 48.41% in the controls, and 13.34%, 45.55% & 41.11% in AF subjects, respectively ($P = .038$) (Table 2). There was statistical difference in genotype distribution between cases and controls and as well as significant difference in A allele frequency of *IL-10(-592A/C)* was observed between the two groups (36.11% vs 27.77%, $P = .065$) (Table 3). The frequency of the A allele in the AF group was significantly higher than that in the control group. Compared with the CC genotype, the AA genotype had a 1.3-fold increased risk of AF. In multivariate analyses, the *IL-10(-592A/C)* variant was associated with a significant predisposing effect on AF after adjusting for related risk factors and the odds ratio for case with AA genotype was 1.237 (95% CI:1.594-10.898, $P = .025$) (Table 4).

4 | DISCUSSION

IL-10 is a major anti-inflammatory cytokine that downregulates cell-mediated immune responses and cytotoxic inflammatory responses.[13] Our study demonstrated a genetic association between the *IL-10(-592A/C)* and post-OP AF in a north Indian population. The findings suggested that A allele confers a higher susceptibility to AF in the north Indian ethnicity.

TABLE 2 Distribution of *IL-10-592A/C* genotypes in cases and controls

Genotype	CC	AC	AA	*P*
Post-OP AF (cases)	37 41.11%	41 45.55%	12 13.34%	.0385
Sinus (controls)	61 48.41%	60 47.61%	5 3.96%	

Post-op AF, postoperative atrial fibrillation; Sig, $P \leq 5\%$ significant.

TABLE 3 Distribution of IL-10-592A/C alleles in cases and controls

Allele frequency	A	C	*P*
Post-OP AF	65 36.11%	115 63.89%	.904
Sinus	70 30.63%	182 69.37%	.036

Post-op AF, postoperative atrial fibrillation; Sig, $P \leq 5\%$ significant.

Previous studies showed the association of the inflammation contributes to the frequent occurrence of AF after the cardiac surgery.[13] Inflammatory stimuli may lead to structural remodeling of atria that promotes progression and persistence of AF.[14] Watanabe and co-authors found that elevated levels of CRP existed in persistent AF.[15] Psychari and colleagues showed that CRP and interleukin *IL-6* were positively related to left atrial diameter, and thus a significant relationship existed between *IL-6* levels and AF.[16] Therefore, to achieve a similar goal of AF predictor a genetic study was designed by selecting a gene *IL-10(-592A/C)* in north Indian population.

In the polymorphism studies, the frequency of variant A allele in AF group of different population were 83.33% in Chinese Han,[17] 67.00% in southern Chinese, 62% in Korean, 67.20% in Japanese and 21% in Caucasians population.[18–20] In our study of north Indian AF patients, the frequency of A allele is also higher than the control group (36.11% vs 30.63%, $P = .904$). The genotype frequency of AA in cases shown the significant difference ie, genetic variant has influence in the development of abnormal heart rhythm (13.34% vs 3.96%, $P = .0385$). The incidences of post-OP AF were higher in patients of VHD surgery (60%) than the CABG surgery patients (40%). In an off-pump CABG study AF incidence was 28%[5] but in our study, it is 40%. This might be because the incidence of post-OP AF depends on its definition and modality of diagnosis. Advancing age has been consistently reported as a risk factor for post-OP AF but in our data, it has shown that younger population of the average age of 40 years are in cases than the control group (44.3 vs 52.55 years, $P = .027$) respectively. In the present study, logistic regression analysis revealed that age was significantly associated with the development of post-OP AF. The time of extubation tended to be longer in patients with post-OP AF than those without ie, 19.35 vs 18 hours respectively. Postoperative AF usually occurs within the first postoperative day and was also observed on postoperative days 2nd, 3rd and 4th days, for evidence daily ECG was collected. The nor-cardiac morbidity such as diabetes has shown a significant result (95% CI: 1.323-4.423, $P = .004$). Other cardiac and noncardiac factors did not influence much in AF patient's odds ratio such as in smoking (95% CI: 0.339-1.560, $P = .413$), tobacco (95% CI: 0.069-1.104, $P = .069$), hypertension (95%CI: 1.879-6.497, $P = .000$), thyroid (95% CI: 0.053-1.372, $P = .114$), sleep apnea (95% CI: 0.683-3.274, $P = .314$).

5 | CONCLUSIONS

In conclusion, gene *IL-10(-592A/C)* has a significant role in the development of post-OP AF. The largest benefit that will be drawn from all these data is the much improved understanding of the disease, how it is initiated and how it chronifies. Once the preliminary data is obtained, the development of better therapeutic and preventive measures will be a possibility. The data support that *IL-10(-592A/C)* polymorphism is associated with AF and the A allele has increased the risk for AF in north Indian population. Therefore, *IL-10 (-592A/C)* could be a biomarker in future. If study is further carried out in all parts of India then an important conclusion could be established.

Study has some limitations regarding controls like controls were hospital based, but not the population-based. Secondly, we could not exclude the presence of previous asymptomatic AF in the control group because these conclusions were based solely on the medical history and interviews with the participants. The relatively limited population size restricts the generalizability of our results. Thirdly, except *IL-10*, other inflammatory cytokines, might be measured to

TABLE 4 Multivariate analysis for *IL-10-592A/C* polymorphism according to logistic regression model in north Indian population

Covariate	B	SE	WALD	DF	Sig	EXP
AC	1.359	0.572	5.642	1	0.017	3.892 (1.269-11.938)
AA	1.237	0.569	4.998	1	0.025	3.571 (1.170-10.898)
Diabetes	0.883	0.308	8.223	1	0.004	2.419 (1.323-4.423)
Smoking	−0.318	0.389	0.669	1	0.413	0.727 (0.339-1.560)
Tobacco	−1.288	0.708	3.313	1	0.069	0.276 (0.069-1.104)
Hypertension	1.251	0.317	15.6	1	0.000	3.494 (1.879-6.497)
Thyroid	−1.312	0.831	2.494	1	0.114	0.269 (0.053-1.372)
Sleep apnea	0.402	0.400	1.014	1	0.314	1.496 (0.683-3.274)

EXP, odds ratio; Sig, $P \leq 5\%$ significant.

clarify possible causative mediators. Our limitations of case-control studies and the complex nature of genetic susceptibility for chronic degenerative diseases, the prospective and interventional clinical studies with larger sample size are required to be conducted in more ethnic groups to confirm our observations.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The Institutional Ethics Committee of the Sanjay Gandhi Postgraduate Institute of medical sciences approved all procedures involving human genome use. Written informed consent for this investigation was obtained from all participants.

CONSENT FOR PUBLICATION

Written informed consent for this publication was obtained from all participants.

AVAILABILITY OF DATA AND MATERIALS

Data obtained from history of patients and orally.

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CONFLICT OF INTEREST

Authors declare no conflict of interests for this article.

AUTHORS' CONTRIBUTIONS

All authors read and approved the final manuscript.

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