Section A-Research paper
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Study of Nuclear receptor (NR4A3) gene expression levels of atrial appendagetissues (Right & Left) in patients with structural heartdiseases in Telangana population.

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Abstract:

BACKGROUND:

INTRODUCTION

The Atrial appendages are remnants of the original embryonic atrium formed during the third week of gestation.(1)Both the right and left appendages are trabeculated, with muscle bars largely running parallel to each other giving a comb-like appearance (hence termed pectinate muscles), but these are less pronounced on the left.(2)

Left atrial appendage locatedon the lateral wall of heart close to the left circumflex artery, which is relevant for surgical occlusion.(2)Right atrial appendage is broad and triangular with a wide junction. (3)

Atrial fibrillation (AF) itself and underlying heart diseases lead to changes in atrial function and structure. Often it is difficult to distinguish the contribution of AF to these multiple processes known as atrial remodeling.(4,5)Molecular research of structurally changed human atria has been preferably focused on various ion channels (6,7)and proteins involved in calcium homeostasis,(8,9) while the links between different pathways involved in the pathology of AF

have so far not been investigated.[10]This has stimulated the search for novel approaches that could help to find the relationship between the underlying conditions.Nuclear receptors (NRs) are master regulators of a plethora of cellular and biological processes such as cell proliferation, migration, apoptosis, metabolism, and differentiation. Recently, NRs of the NR4A subfamily have emerged as key players in the pathophysiology of heart diseases [11]

AIM: Study of Nuclear receptor NR4A3 gene expression levels of atrial appendagetissues (Right & Left) in patients with structural heart diseases in Telangana population.

OBJECTIVES:

1)To evaluateNuclear receptor NR4A3 gene expression levels in atrial appendage tissue samples in control and cases byusing RT-PCR & cDNA microarray method.

2) To compare Nuclear receptor NR4A3 gene expression levels between control and cases of atrial appendage tissue samples byusing RT-PCR & cDNA microarray method.

MATERIALS AND METHODS:

Type of study design: Case Control study

Study setting: Patients were enrolled in the Cardiovascular thoracic Department of Virinchi Hospital, Hyderabad.

- Inclusion criteria: Those Patients who had structural deformities in heart and consented to participate in the study.
- Exclusion criteria: Do not give informed consent , Idiopathic Ventricular Tachycardia (Non structural heart disease), Coronary artery disease
- Ethics consideration : Approved by Institutional ethical committees of :-

A)Vinayaka Missions Medical College and Hospital Karaikal ,VMRF DU Salem.

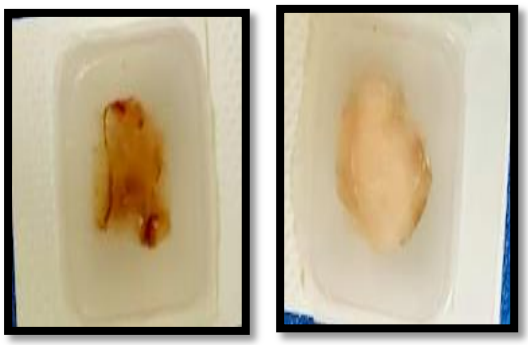
B)Virinchi Hospital, Hyderabad.

Study participants: Written Informed consent obtained.100 samples of atrial appendages collected from patients who underwent cardiac bypass surgery, mitral valve replacement or left atrial myxoma excision and divided into two groups.

- **Case/ Group1** :-Atrial appendage were collected from 50 patients, (31 Males and 19 Female with age group range 35 to 70 years) with structural heart disease with history of Atrial Fibrillation and who came for open heart surgery, Mitral valve replacement surgery and Left atrial myxoma.Out of 50 atrial appendage tissue samples :- 47RAA and 3LAA.
- **Control / Group2** :- Atrial appendage were collected from50 patients, (43 Males and 07 Females with age group range 35 to 70 years) with structural heart disease without history of Atrial fibrillation and who came for open heart surgery, Mitral valve replacement surgery and Left atrial myxoma.Out of 50 atrial appendage tissue samples:- 45RAA and 5LAA

Study Parameters :-

1) To evaluateNuclear receptor NR4A3 gene expression levels in atrial appendage tissue samples incontrol and casesbyusing RT-PCR & cDNA microarray method.
2) To compare Nuclear receptor NR4A3 gene expression levels between control and cases of atrial appendage tissue samples byusing RT-PCR & cDNA microarray method.
3) **Methodology:**
   - Excision: -Immediately after excision, atrial appendage samples were frozen in liquid nitrogen and stored at –80 degree celsius until RNA isolation.
   - RNA isolation and then RT-PCR and cDNA microarray analysis had been done .



Right atrial appendage Left atrial appendage

- **STASTICAL ANALYSIS:** All RT PCR data and cDNA-array were normalized to the set of stable expressed genes and statistically analysed using Mann-Whitney U test to evaluate the significance of differences between gene expression mean values for Group 1&2.All values were expressed as mean+_ SD. P value < 0.05 considered as statistically significant.
- **RESULTS:** RT-PCR & cDNA analysis were performed for 100 atrial appendage tissue samples from patients with structural heart disease with AF as Case / Group1 and Control / Group2.25 genes with altered expressions were observed. Out of 25 genes 13 genes shows more pronounced expressions between cases and control group by RT-PCR analysis (fig.1) .GADPH gene used to normalise RT-PCR data.All these genes were downregulated in group1 patients as compared to Group2, NR4A3 shows significant expression levels.

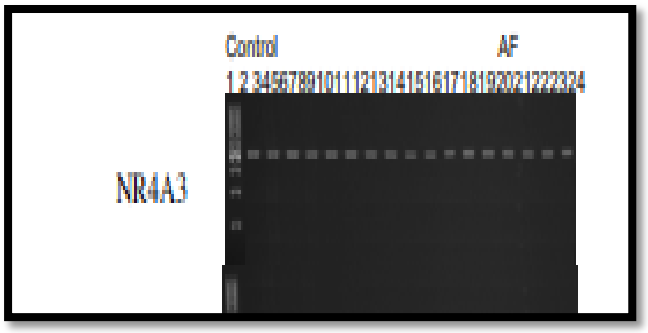


FIGURE no.1 :- Electrophoregrams of PCR products of NR4A3 differentially expressed gene.

- Lanes1-10 :- RT PCR products synthesized on mRNA from AA of control subjects.
- Lanes 11-22 :- RT PCR products synthesized on mRNA from AA of AF patients.
- Lane 23 is negative PCR Control.
- Lane 24 is 100bp DNA ladder.
- Gene name is indicated.

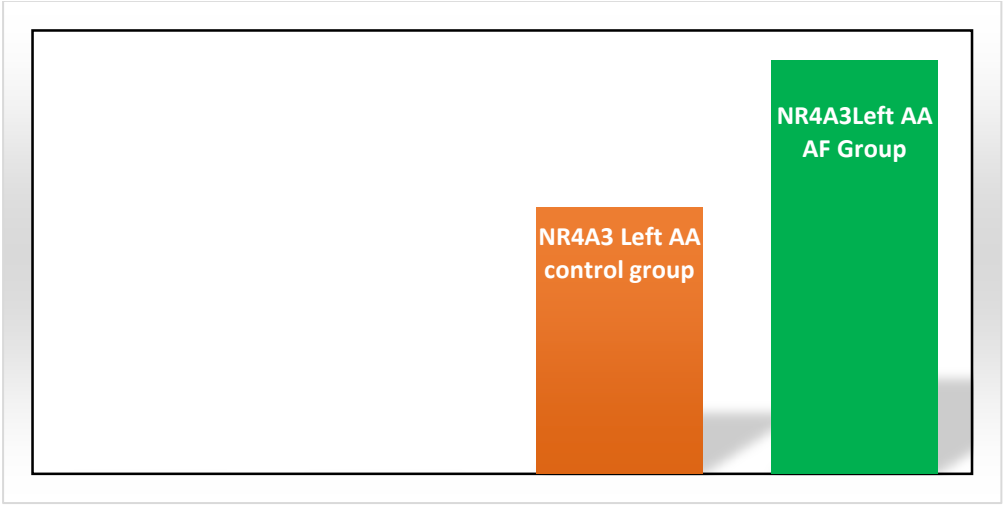


FIGURE no.2 :- Histogram of averaged NR4A3 gene expression level in control and AF groups of Left AA.The data are plotted after densitometrical analysis of the electrophoregrams in FIGURE 1. (AA-atrial appendage, AF-atrial fibrillation)

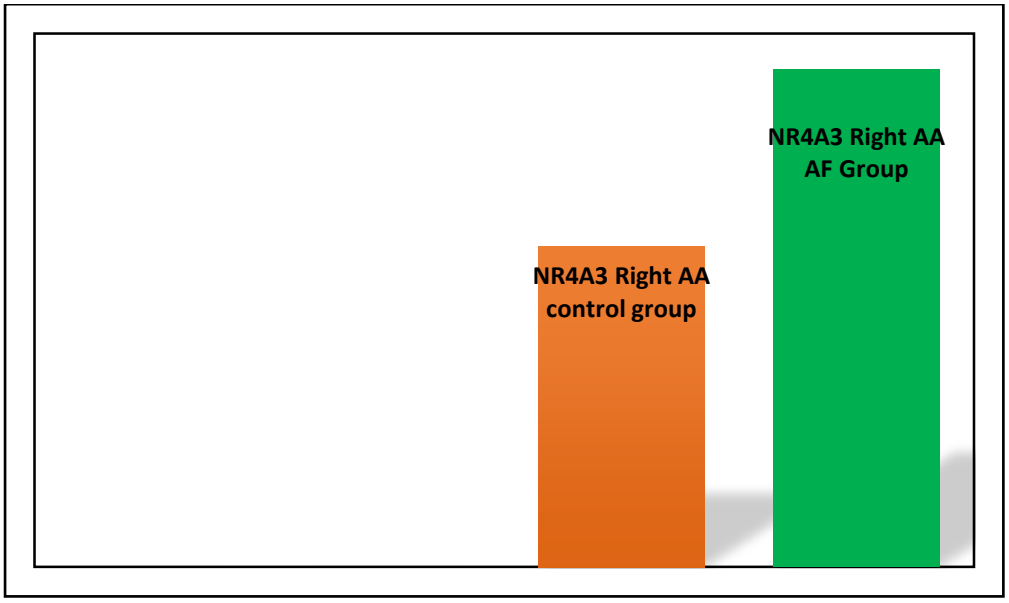


FIGURE no.2 :- Histogram of averaged NR4A3 gene expression level in control and AF groups of Right AA.The data are plotted after densitometrical analysis of the electrophoregrams in FIGURE 1. (AA-atrial appendage,AF-atrial fibrillation)

Discussion: Using RT-PCR analysis and cDNA microarray technique , we have identified NR4A3 gene discriminating between pathologically altered and control atrial tissues. NR4A3 genewas downregulated in AF patients compared with the control group.

CONCLUSION : By Identifying the functional importance of NR4A3 gene in the development and maintenance of structural heart disease with or without Atrial fibrillation is important, so that we can know underlying molecular mechanism and we can identify new therapeutic strategies.

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