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Tumour Necrosis Factor Receptor Type II Gene Polymorphism in Coronary Heart Disease

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Abstract **Original Research Article**

Introduction: Coronary Heart Disease (CHD) is the leading cause of mortality and morbidity worldwide. Tumour Necrosis Factor type 2(TNFR2) is increased in patients with Coronary Heart Disease and Peripheral Vascular Disease. So genetic polymorphism in TNFR2 may indicate susceptibility to CHD. ***Aims & objectives:*** The study aim at whether TNFR2 gene polymorphism is the cause for differences in the Coronary Heart Disease susceptibility among the population. ***Materials &methods*:** The study sample comprised of 158 cases of Coronary Heart Disease and 149 age and sex matched controls. Patients were chosen from the Department of Cardiology, Stanley Medical College and Hospital. Subjects with diabetes, hypertension, hypothyroidism and autoimmune diseases were excluded from the control group. 5ml of peripheral venous blood was withdrawn for DNA extraction and lipid profile. 242 bp fragment of TNFR2gene was amplified, followed by restriction digestion.Lipid profile was done by chemical method. ***Results & observation*:** The percentage of the MM genotypes of TNFR2 gene polymorphism in patients with Coronary Heart Disease was found to be 39% and 21%in controls (p value = 0.00) (odd's ratio 2.46).RM genotype was found in 79% controls and 57% cases(p value 0.00),(Odd's ratio 0.36) Among the three genotypes, MM homozygote showed an increased risk**.** ***Conclusion:*** We conclude that MM genotype is an independent risk factor for Coronary Heart Disease.
Keywords: Coronary Heart Disease, TNFR2, polymorphism, restriction digestion, genotype, lipid profile.

INTRODUCTION

Coronary Heart disease is the leading cause of mortality and morbidity worldwide and is rapidly increasing in incidence in nations undergoing industrial development[1].Cardiovascular disease is now the most common cause of death worldwide replacing infection. Coronary Heart Disease(CHD) is responsible for about one-third of all deaths in individuals over age 35[2].The major cause of CHD is atherosclerosis. "Atherosclerosis is a lipid driven chronic inflammatory disease of the arterial wall leading to multifocal plaque development at sites characterised by low velocity flow and pre-existing intimal thickenings[3].Tumor Necrosis Factor or TNF α, a proinflammatory cytokine stimulates the expression of endothelial genes which promote atherosclerosis.TNFα exerts its action through two receptors, TNFR1 and TNFR2. Studies have shown that TNFR2 is increased in patients with Coronary Heart Disease and Peripheral Vascular Disease. So genetic polymorphism in or near TNFR2 may indicate susceptibility to CHD[4].

Aim and objectives of the study

The aim of the study was to find if TNFR2 gene polymorphism is the cause for differences in the Coronary Heart Disease susceptibility among different populations, if there is any correlation of any specific genotype with expression of the receptor and to find out the association between risk factors and the disease.

MATERIALS AND METHODS

The present study was done in Stanley Medical College between November 2011 to November 2012. It was carried out in two groups, apparently healthy controls and patients with angiographically confirmed diagnosis of Coronary Heart Disease. The study was approved by the Institutional Ethical Committee of Stanley Medical College.

Study population

The study sample comprised of 158 CHD patients. Patients were chosen from the Department of Cardiology, Stanley Medical College and Hospital. The diagnosis of CHD was established by ECG, cardiac markers and angiography. Clinical history such as diet, diabetes, hypertension, smoking, alcoholism were also

collected.149 Controls were recruited from Master Health Check up clinic during their visit to this hospital. Healthy, age-matched people, without a family history of CHD were selected to serve as the control group.

Subjects with diabetes, hypertension, hypothyroidism, autoimmune diseases were excluded from the control group.

Sample collection

5ml of peripheral venous blood was drawn under sterile conditions with disposable syringes from all the cases and controls of the study and 2ml transferred to EDTA tube and mixed thoroughly. The EDTA tube was centrifuged to get the buffy coat for DNA extraction. The remaining 3ml of blood was centrifuged at 3000rpm for ten minutes to separate the serum.

Buffy coat was separated by centrifugation of EDTA tubes at 2000 revolutions for 20 minutes. DNA extraction of the samples were done by kit and chemical methods. Extracted DNA was identified by 1% agarose gel electrophoresis and comparison with a known molecular weight 1kb DNA ladder as depicted in Fig.(1).242 bp fragment of TNFR2 gene was amplified using Forward primer-5' ACTCTCCTATCCTGCCTGCT3' and Reverse primer-5'TTCTGGAGTTGGCTGCTGCT3'. Amplification was carried out in Bioneer, My Genie 96 Global Partner thermal cycler with the following cycling conditions. Initial denaturation - 95^0 C -5min followed by 30 cycles of Denaturation - 94^0 C – 1min, Annealing- 64^0 C – 1min, Extension- 72^0 C – 2min and Final extension at 72^0 C - 5 min.

Amplified product – amplicons of 242 bp was identified by 2% agarose gel electrophoresis by comparison with a known 100bp DNA ladder.

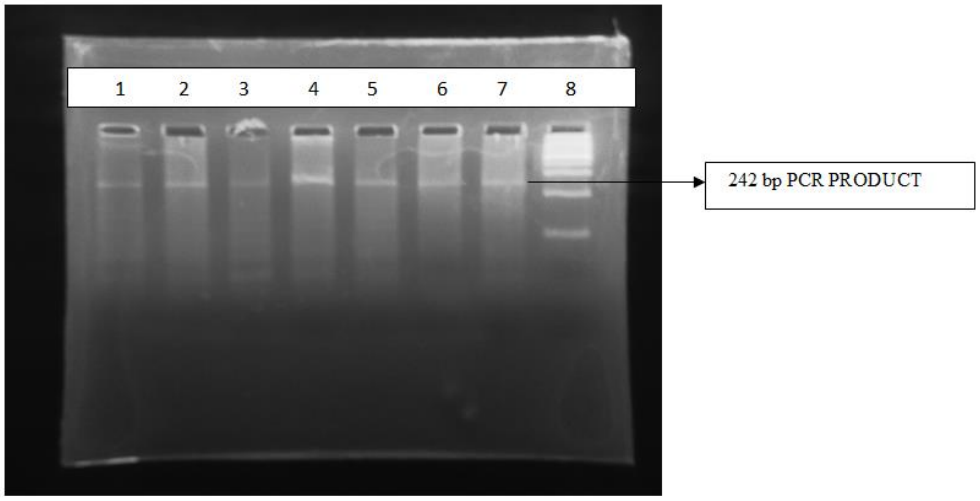


Fig-1: Agarose gel electrophoresis of 242 bp pcr product

LANE 8-100bp DNA Ladder with extension to 1500 bp, LANE 1,2,3,4,5,6-242bp PCR product.

Agarose gel electrophoresis

PCR product was run on 2.0% agarose gel. TNFR2 polymorphism was detected by digestion of the PCR amplified product with the Nla III restriction enzyme followed by run in 3.0% agarose gel electrophoresis.

Principle of Nla III enzyme digestion

The restriction site is absent in R allele, hence will yield a 242bp fragment. M allele has the restriction site, hence gets cleaved to give 133bp and 109bp fragments.Heterozygous individuals (RM) have 242 bp, 133bp,109bp fragments. Analysis was done using a 100bp DNA ladder.

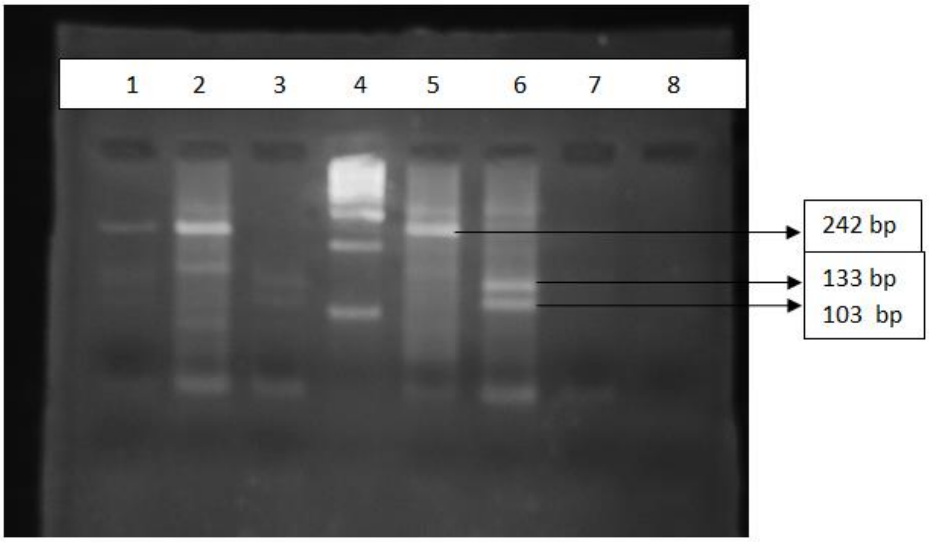


Fig-2: Agarose gel electrophoresis of restriction digested products

LANE 4 – 100bp DNA ladder with extension to 1500 bp; LANE 1,2 –RM, LANE 3,6- MM, LANE 5-RR

Total cholesterol was estimated by CHO-PAP Method,Triglycerides by GPO-PAP Method, HDL - Direct Method ,LDL was calculated by Friedwald formula. Blood Sugar was done by GOD-POD Method.

Statistics

Age and lipid profile levels between CAD patients and control subjects were compared using student 't' test.Genotype distribution and risk factor status between patients and controls were compared with χ^2 using 2*2 contingency table. Odd's ratio and confidence interval were used find the association between smoking, alcohol, obesity, lipid profile and the disease.

RESULTS

Table-1: Characteristics of study and control groups

	Patients	Controls
Mean Age in years (Range)	52.92 (37-72)	52.95(20-75)
Sex (M/F)	123/35	112/37
Smoking	80	50
Alcohol	75	47
Diabetes	29	0
Hypertension	109	0
Total Cholesterol(mg/dl)	207.85 ± 47.52	178.30 ± 41.38
Triglycerides(mg/dl)	186.13 ± 99.31	148.07 ± 50.25
LDL(mg/dl)	128.98 ± 42.19	117.29 ± 38.20
HDL(mg/dl)	41.54 ± 9.07	45.52 ± 9.21
BMI	26.51 ± 4.36	23.65 ± 4.26

Table.1 shows the mean age, sex, number of smokers, alcoholics, diabetics, hypertensives among patients and controls. Total cholesterol, TGL, HDL, LDL, BMI values are given as mean ± SD.

There was no significant difference with respect to age in CHD patients and controls, but the Cholesterol, TGL, HDL, LDL values differ significantly between the two groups. This implies that increase in cholesterol, TGL,LDL and decrease in HDL are risk factors for the disease.

Table-2: Association of Risk Factors and incidence of CHD

Risk Factors	OR	CI	P
Smoking	2.03	1.28 - 3.22	0.00*
Alcohol	1.96	1.23 – 3.12	0.00*
Obesity	5.15	2.55 – 10.41	0.00*
MM Genotype	2.46	1.48-4.09	0.00*

*p <0.05-considered significant

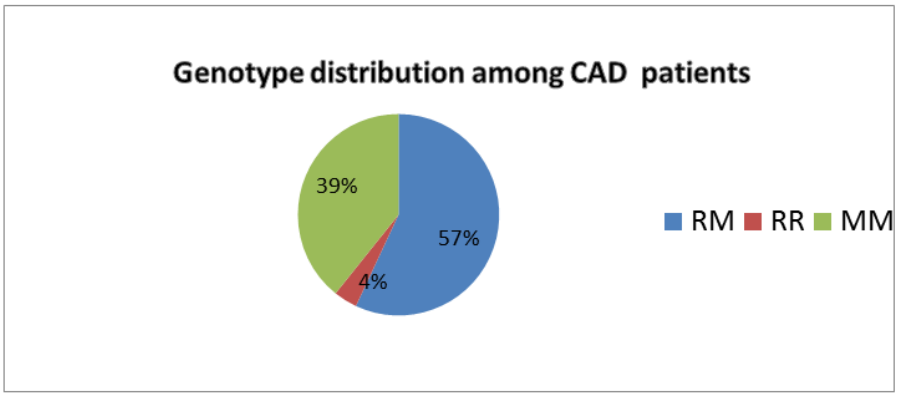
Table 2 shows the relationship of riskfactors with CHD. There is statistically significant difference in risk factor states between the two groups, p<0.05. Alcohol OR-1.96(95% CI 1.23-3.12) and Smoking OR-2.03(95% CI 1.28-3.22) were associated with a two fold increase in CHD incidence. Obesity OR-5.15(95% CI 2.55-10.41) was associated with five fold increase in the incidence of the disease. MM genotype was a risk factor for the development of CHD OR-2.46(95% CI 1.48-4.09).

Table-3: Genotype distribution of TNFR2 gene between patients and controls

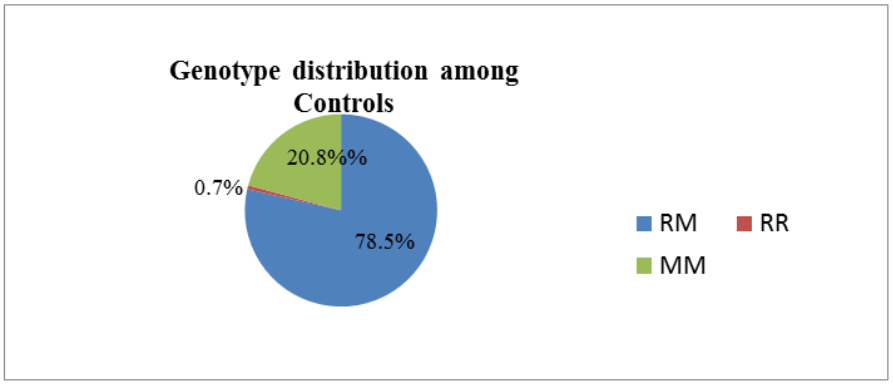
Genotype	Patients n (%)	Controls n (%)	OR	CI	P
MM	62 (39%)	31(20.8%)	2.46	1.48-4.09	0.00*
RM	90 (57%)	117 (78.5%)	0.36	0.22-0.6	0.00*
RR	6 (4%)	1 (0.7%)	5.84	0.7-49.1	0.12

(χ^2 value – 17.09; P < 0.0001*) ; * p < 0.05- considered significant

Table 3 shows genotype distribution between CAD patients and control subjects. MM genotype was found to be more common in patients (39%) than controls (21%). RM genotype occur frequently in controls (79%) than CAD patients (57%). There is statistically significant difference in genotype distribution (MM & RM) in patients and controls, p<0.05. MM genotype was associated with increase in incidence of CHD(67%) with OR-2.46(95% CI 1.48-4.09) and RM genotype was protective(43%) with OR-0.36(95% CI 0.22-0.6).



Pie chart showing genotype distribution among chd patients



Pie chart showing genotype distribution among control subjects

There was statistically significant difference in allele frequency between the two groups. The M allele was more common in patients(68%) compared to Controls(60%). It may be considered that M allele was a risk factor for CHD (OR-1.39; 95% CI 1.002-1.941, p<0.05). There was no significant association between any of the genotypes and DM in CHD patients. There was no difference in genotype MM between patients with diabetes and without diabetes.There was no statistically significant difference between the genotype distribution and hypertension in CHD patients,p>0.05.There was no statistically significant increase in MM genotype in patients with hypertension.

DISCUSSION

Tumour Necrosis Factor α is a proinflammatory cytokine involved in the pathogenesis of wide range of inflammatory diseases. TNFα exerts its actions via TNFR1 and TNFR2. TNFR2 is

predominantly expressed in neuronal cells, endothelial cells and cardiac myocytes. The present study attempts to investigate the relationship between TNFR2 gene Nla III polymorphism and Coronary Heart Disease. In CHD patients, a microsatellite marker with 5 alleles in intron 4 of this gene has been found to be associated with the disease(5). TNFR2 gene is localized on chromosome 1p36.2 and comprises of 10 exons and 9 introns(6) that span a region of 26 kb[7].The genetic segment of present interest is located in exon 6 at codon position of 196.

The change in nucleotide sequence at codon 196 ATG instead of AGG creates a restriction site for the endonuclease enzyme Nla III. The people with M (Methionine) allele will have this restriction site being acted upon by this enzyme. The people with R(Arginine) allele will not have this restriction sequence for the enzyme.We found that M allele having the restriction site for the enzyme was increased in CHD patients(68%) than controls(60%). In controls, R allele was increased(40%) compared to patients(32%). OR for M vs R allele was 1.39 (95% CI 1.002-1.941), $p<0.05$. This shows that M allele is a risk factor for the development of CHD.

Homozygotes with MM genotype(67%) were more prone for the disease than the heterozygous RM genotype(43%). OR for MM genotype vs Others was 2.46(CI- 1.48-4.09), $p<0.05$, shows that this genotype was associated with two fold increased risk for development of Coronary Heart Disease.

There was negative association for RM genotype with the disease. It implies RM genotype is protective against CHD. Infact increased TNFα levels have been found in patients with premature Coronary Heart Disease[8]. TNF is found to be associated with metabolic and cellular perturbations of the atherosclerotic process[9].

CONCLUSION

From this study, we conclude that MM Genotype may be an independent risk factor for the development of Coronary Heart Disease. RM Genotype is protective against development of Coronary Heart Disease. Smoking, Alcoholism, Obesity are independent risk factors of the disease. Diabetes and Hypertension does not have influence on the genotype distribution.

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