

## Association of C-262 polymorphism in catalase gene with rheumatoid arthritis

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### Abstract

**Objectives:** To evaluate the association of C-262 polymorphism in Catalase gene (CAT) with Rheumatoid Arthritis.

**Method:** The comparative cross-sectional study was conducted at the Department of Biochemistry and Molecular Biology, Army Medical College, Rawalpindi, in collaboration with the Rheumatology Department, Pak Emirates Military Hospital, Rawalpindi, Pakistan, from January to December 2020, and comprised Deoxyribonucleic acid extraction of samples. Samples in group I belonged to diagnosed rheumatoid arthritis patients of either gender aged 30-60 years who were on disease-modifying anti-rheumatic drugs. Group II had an equal number of healthy controls. The promoter region of the CAT gene having the polymorphic segment was amplified through polymerase chain reaction, and its products were then subjected to restriction fragment length polymorphism for the analysis of polymorphic region of the CAT gene. Genotypic frequency equilibrium and the association of polymorphism with rheumatoid arthritis was checked. Also, association between fasting lipid profile and haemoglobin was assessed. Data was analysed using SPSS 22.

**Results:** Of the 60 samples, 30(50%) belonged to each of the two groups. The mean age was 44.90±10.50 years (range: 30-60 years). Overall, there were 34(56.7%) males and 26(43.3%) were females. Two alleles and three genotypes of the polymorphism was detected. The frequency of CC genotype was higher in group I 23(76.6%), but no association of any of the genotype of polymorphism was found significant ( $p \leq 0.05$ ). Haemoglobin and lipid profile levels were significantly different in the two groups ( $p \leq 0.05$ ).

**Conclusion:** There was no significant association found between C-262 polymorphism in CAT gene and rheumatoid arthritis.

**Keywords:** Rheumatoid arthritis, CAT gene, C-262T polymorphism, Restriction fragment length polymorphism. (JPMA 72: 2404; 2022) DOI: <https://doi.org/10.47391/JPMA.3451>

### Introduction

Rheumatoid arthritis (RA) is a systemic, chronic inflammatory and autoimmune disease of joints that presents with pain, inflammation and decreased mobility. In Greek the term RA is used for inflamed and watery joints. Prevalence of RA till 2019 was approximately 1.5% worldwide.<sup>1</sup> Regional prevalence of RA for countries of poor or moderate socio-economic status is 0.40%.<sup>2</sup> RA prevalence in Karachi, Pakistan, in 2015 was 26.9%.<sup>3</sup>

The mainstream evidence that is derived from tissue analysis, genetics, models and clinical studies suggest an immune-mediated aetiology of RA characterised by the presence of auto reactive antibodies. The heritability of RA has been estimated at 60%,<sup>4</sup> implying that 40% of the variance in developing RA might be explained by environmental risk factors. In addition, genetic factors play a major role in the severity of joint damage as the heritability of the severity of joint damage has been

estimated to be 45-58%.<sup>5</sup> Genes play a vital role in the development and progression of RA.

A pre-RA phase that lasts from months to years is characterised by oxidative stress (OS) and altered metabolism. Free radicals, like reactive oxygen species (ROS), have high reactivity with biological macromolecules, such as deoxyribonucleic acid (DNA), forming neoepitopes that eventually results in production of polyspecific autoantibodies, resulting in autoimmunity. Thus, OS is one of the probable causes of development of RA.<sup>6</sup>

Similarly, a pathological hallmark of RA is the augmented production of free radicals. A fivefold increase in mitochondrial ROS production is also seen with RA.<sup>7</sup> ROS are indirectly associated in joint damage because of their role as secondary messengers in immunological and inflammatory cellular responses in RA. ROS can degrade joint cartilage directly by attacking and inhibiting synthesis of proteoglycans. Lipoperoxidation, oxidative damage of hyaluronic acid, oxidation of lipoproteins and protein oxidation resulting in carbonyl increment have also been demonstrated in RA. Therefore, ROS and OS play a vital role in the progression of RA as well.<sup>8</sup>

Catalase, due to its high turnover frequency, is considered one of the first-line defence mechanisms in combating

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and suppressing the formation of free radicals. Catalase plays a pivotal role in pathogenesis and severity of a heterogeneous disease like RA. Importantly, with respect to outcomes in RA, low catalase levels are responsible for high inflammation in RA. Catalase activity was significantly lowered in RA patients compared to controls in a study.<sup>9</sup>

Catalase enzyme is encoded by CAT gene that has been mapped to the 11th chromosome, band p13. CAT gene, a collaborative gene, does not contain any TATA box and initial element sequence. It has high GC content in promoter and a core promoter. The gene has three CCAAT and three GC boxes in the promoter region. This shows that it has multiple initiation points of transcription.<sup>10</sup>

Some genetic polymorphism might evoke changes in expression or activity of the enzyme. CAT 262 polymorphism, a single nucleotide polymorphism (SNP), consists of T nitrogen base instead of the usual C at -262 position in the 5' untranslated region (UTR). It is a promoter region polymorphism affecting transcription factor binding, thus altering the gene expression and ultimately the oxidative status of cells and their microenvironment.<sup>11</sup> It has been found that CAT 262 polymorphism is associated with altered catalase activity in erythrocytes.<sup>12</sup> The polymorphism has three genotypes CC, CT and TT and two alleles 'C' and 'T' as reported by the National Centre of Biotechnology Information (NCBI).<sup>13</sup>

The current study was planned to identify additional genetic risk factors for clinical RA and to increase the understanding of processes underlying RA development.

## Patients and Methods

The comparative cross-sectional study was conducted at the Department of Biochemistry and Molecular Biology, Army Medical College (AMC), Rawalpindi, in collaboration with the Rheumatology Department, Pak Emirates Military Hospital (PEMH), Rawalpindi, Pakistan, from January to December 2020. After approval from the AMC ethics review committee, the sample size was calculated using the World Health Organisation (WHO) calculator for two proportions, by taking the proportion of CC as 95.1% and CT+TT as 4.9% in RA patients,<sup>14</sup> with 95% confidence interval (CI) and 5% margin of error. Additional subjects were enrolled for better generalisability of the findings. The sample was raised using non-probability convenience sampling technique from among patients visiting the PEMH Rheumatology Department.

Those included were diagnosed RA patients of either gender aged 30-60 years who were on disease-modifying anti-rheumatic drugs (DMARD) in group I, and an equal

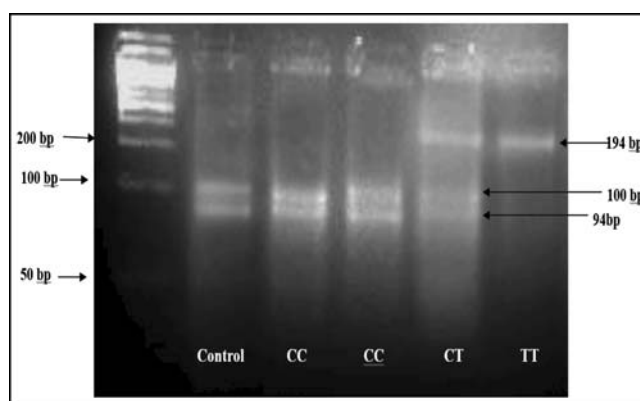
number of healthy controls in group II. Patients of other chronic illnesses, patients of any other type of arthritis, and RA patients not on DMARD therapy were excluded. After taking written informed consent from each subject, demographic and clinical data was recorded using a questionnaire.

DNA extraction in most cases was done using Thermo Scientific Genomic DNA Purification Kit (#K0721) and in some cases by the phenol chloroform method. Extracted DNA was then stored at -20°C. Genotyping of gene was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Primers of the targeted region were constructed using Primer 3 software, with the sequence retrieved from the NCBI database.<sup>13</sup>

Forward primer: 5'-ATTCCGTCTGCAAACTGGC-3'

Reverse primer: 5'-GCCTGAAGACCGGAGATACC-3'

The promoter region of CAT gene containing -262 C/T polymorphic segment was amplified by PCR. The total volume of each PCR reaction mix was 25µl. Each cycle included reaction that first denatured the template strand at 94°C for 35sec, annealing of primers was done at 58.7°C for 35sec, and then the primer was extended at 72°C for 35sec. The final elongation was carried out for 8min at 72°C. For each sample, 35 cycles of PCR amplification yielded favourable fragment size. Agarose gel electrophoresis (AGE) stained with ethidium bromide for checking of amplified products was performed on 2% matrix. An amplicon of 194 base pairs (bp) was obtained. Amplicons (194bp) were then digested with 10U of SmaI (10U/µL) (Thermo Scientific ER 0662) at 25°C for 4hrs and analysed on 4% AGE stained with ethidium bromide. The 'T' allele was the undigested allele, giving 194bp segment,



**Figure-1:** Catalase (CAT) 262 C/T genotyping by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). M= 50bp ladder, lane 1= Control showing C/C genotype (100, 94bp), lane 2 = CC genotype (100, 94 bp), lane 3 = CC genotype (100,94bp), lane 4 = CT genotype (194, 100,94bp), lane 6 (194bp).

when the 'C' allele was digested and gave two fragments of 100 and 94bp (Figure-1).

Data was analysed using SPSS 22. The quantitative variables passed the preliminary screening for normality assessment. Shapiro Wilk normality test was used to check normal distribution of the variables ( $p=0.3$ ). Descriptive statistics were calculated for quantitative variables that were distributed normally, and were then compared using independent t-test. Hardy Weinberg equilibrium (HWE) principle was assessed by using chi-square test with one degree freedom to find the association of polymorphic frequencies in the groups.  $P \leq 0.05$  was considered statistically significant.

**Results**

Of the 60 subjects, 30(50%) belonged to each of the two groups. The overall mean age was  $44.90 \pm 10.50$  years (range: 30-60 years), and there were 34(56.7%) males and 26(43.3%) females with an equal gender distribution between the groups. Of all the participants, 42(70%) were from Punjab, 8(13.3%) from Khyber Pakhtunkhwa (KP), 7(11.6%) from Azad Jammu and Kashmir (AJK) and 3(5%) from Sindh. Haemoglobin (Hb) and lipid profile levels were significantly different in the two groups (Table).

DNA extraction of 50(83.3%) samples was done using the Thermo Scientific kit, while 10(16.6%) samples were extracted by the phenol chloroform method. Two alleles and three genotypes of the polymorphism was detected. The frequency of CC genotype was higher in group I 23(76.6%), but no association of any of the genotype of

**Table:** Comparison of biochemical parameters of the study groups.

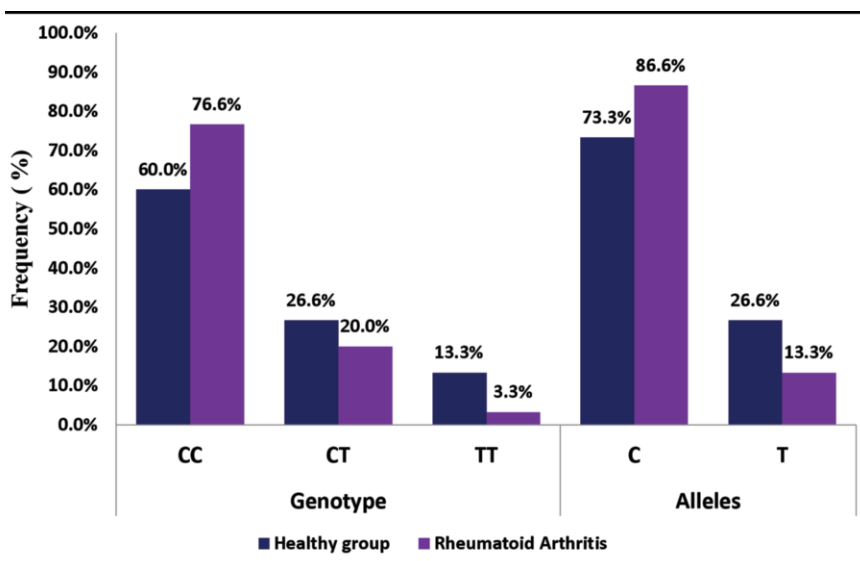
Variables	Healthy Group (N=30) Mean±SD	Rheumatoid Arthritis (N=30) Mean±SD	p Value
TC (mg/dL)	160.86 ± 32.09	191.80 ± 8.89	0.007*
TG (mg/dL)	69.21 ± 47.95	107.88 ± 92.42	0.048*
HDL (mg/dL)	72.69 ± 24.36	42.92 ± 10.05	<0.01*
LDL (mg/dL)	74.24 ± 37.89	122.19 ± 35.96	<0.01*
Hb (g/dl)	13.73 ± 1.89	11.92 ± 1.82	<0.01*

\* $p \leq 0.05$  is considered significant.  
SD: Standard deviation, TC: Total cholesterol, TG: Triglycerides, HDL: High-density lipoprotein, LGL: Low-density lipoprotein, Hb: Haemoglobin.

polymorphism was found to be significant ( $p>0.05$ ). However, HWE test 4.401 implied significant difference in allelic frequencies ( $p=0.043$ ). The wild type CC genotype of the gene showed relatively raised count in the RA group than the controls (Figure-2).

**Discussion**

The current study identified significant difference in lipid profile and Hb levels between the two groups. Anaemia and dyslipidaemia are frequently observed in RA. Inflammatory cytokines play a crucial role in RA. Interleukin 6 (IL6) induces synthesis of hepcidin, which is involved in decreasing intestinal iron absorption, explaining the low levels of Hb in the RA group. Addolorata et al., found a negative correlation between Hb and disease activity in RA.<sup>15</sup> Haematological parameters assessed in a tertiary care hospital of Pakistan showed Hb to be significantly lower in patients with high disease activity which was attributed to upper gastrointestinal complications associated with non-selective non-steroidal anti-inflammatory drug (NSAID) use, resulting in iron deficiency.<sup>16</sup>



**Figure-2:** Comparison of genotypic and allelic frequencies between the two groups. Pearson's chi-square association value was = 0.260 ( $p \geq 0.05$ , non-significant). Odd's Ratio (OR) of polymorphic heterozygous genotype CT = 0.59 and polymorphic homozygous genotype TT = 0.20.

Dyslipidaemia, a recognised risk for cardiovascular disease, is frequently observed in RA. Disturbed total cholesterol was observed in 54% patients in a study<sup>16</sup> which coincided with the current findings. Low high-density lipoprotein (HDL) was the commonest lipid abnormality seen in a study conducted in Karachi on 200 RA patients.<sup>17</sup>

An individual's susceptibility to complex diseases is often determined by certain genetic polymorphisms. SNPs in genes of antioxidant enzymes may have a conspicuous role for inter-

individual differences in maintaining the integrity of the human genome. Role of glutathione peroxidase polymorphism (rs1800668) in Pakistani RA subjects suggested that CT genotype of the studied polymorphism has 1.85 times more risk of RA development.<sup>18</sup>

Several studies have shown that SNPs are dependent upon ethnicity. The current study determined CAT polymorphism in 60 individuals and its association with RA. To the best of our knowledge, the current genetic study is one of the first conducted in Pakistani population. Genotypic variants of the CAT gene have been reported in different populations such as Chinese, South Koreans, Americans, Turkish, Polish and English. The frequency of CC wild type genotype in the studied population was higher, which was similar to the distribution of genotypes seen in South Korean, Chinese and American populations. TT genotype was more widely distributed in Turkish, American Caucasians and Europeans.<sup>19</sup>

Similarly, higher frequencies of the C allele of CAT 262 polymorphism has been reported in Caucasians and Asians; 61-69% and 90-93%, respectively.<sup>20</sup> This coincided with the higher percentages of C allele ( $p=0.8$ ) in the current study's entire population.

Diminished catalase activity has been found in RA patients.<sup>9</sup> CAT C262T polymorphism is intensively studied because the T allele has been associated with decreased catalase activity,<sup>21</sup> resulting in high levels of ROS which increases the probability of RA development. CAT C262T polymorphism did not play any significant role in susceptibility to RA among South Koreans when studied in 474 cases and 400 controls.<sup>14</sup>

C262T polymorphism was not found to be a risk factor for RA development in Pakistani population ( $p=0.26$ ). Large-scale multicentre studies are required to investigate the role of this functional polymorphism.

A Serbian study concluded that TT genotype had 4.36-fold higher chance of juvenile idiopathic arthritis than the carriers of CC and CT genotypes of the studied polymorphism. The genotypic and allelic frequency of CAT 262 C/T polymorphism was significantly different than controls ( $p=0.006$ ).<sup>22</sup> The protective effects are in accordance with the fact that CAT 262 polymorphism is a promoter region polymorphism affecting transcription factor binding, thus altering the gene expression. CAT TT genotype shows lowest activity of catalase, especially in those which are exposed to OS, such as coal dust.<sup>12</sup>

Similarly, influence of polymorphisms in antioxidant genes were investigated in 327 Slovenian RA patients.

CAT -262CC genotype had 2.3-fold probability for high disease activity in RA ( $p=0.007$ ) than CAT- 262 CT or TT genotype, as determined by Disease Activity Score 28 (DAS-).<sup>22</sup> This coincides with the higher frequency of CC genotype with RA group in the current study. However, contrary to the current result, CC genotype was significantly correlated with higher disease activity in RA patients.<sup>23</sup> Thus, T allele has a protective effect, and the finding is in good agreement with the earlier finding that there is a significant positive correlation between number of the T allele and the CAT messenger ribonucleic acid (mRNA) levels, concluding that CAT C-262T polymorphism has higher levels of Catalase.<sup>23</sup>

No CAT gene polymorphism was seen in 200 patients of enteropathic arthritis or patients of inflammatory bowel disease (IBD) in Poland.<sup>24</sup> A study conducted in Iran concluded that CT genotype of CATC262T polymorphism increased the risk of IBD. However, no association of the CAT polymorphism was associated with the risk of IBD.<sup>25</sup>

## Conclusion

There was no association between CAT 262 polymorphism and RA. Moreover, no association was found between CC genotype and RA.

**Disclaimer:** None.

**Conflict of Interest:** None.

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