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### Original article

# Evolving clinical profile of IL-1 $\beta$ , IL-10 and CTLA-4 gene in rheumatoid factor positive Caucasian population.

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#### ABSTRACT

Rheumatoid arthritis (RA), a systemic autoimmune condition, causes joint damage and sometimes extra-articular lesions (cutaneous vasculitis, neuropathy, Felty's syndrome, pericarditis, interstitial lung disease) that may be life threatening. The reason why extra-articular features will develop in rare RA patients is unknown. Our study was aimed to find any disease correlation with respect to a few genetic loci implicated in rheumatoid arthritis. In our study no significant association was observed for IL-1 $\beta$  and IL-10. However CTLA-4 showed polymorphism in RA and controls. In our earlier article we reported polymorphism of HLA-DRB1 in Caucasian population. The study was conducted on 60 patients, where 30 were control and 30 were diseased. All the patients selected for the study were Rf (rheumatoid factor) positive. Therefore the studies need to be conducted on larger group of patients, so that the association can be verified in Caucasian population (Indian scenario).

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### 1. Introduction

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disorder that leads to progressive joint destruction [1]. Hallmarks of RA are joint inflammation, proliferation of synovial cells and attachment and invasion of synovial fibroblasts into adjacent cartilage and bone [2, 3]. Cumulative evidence has indicated that CD4-T cell-mediated autoimmune responses play a critical role in the pathogenesis of RA, in conjunction with activation of B cells and macrophages that infiltrate the synovium [4, 5]. Recent biological therapies against IL-1, IL-10, or TNF- have demonstrated promising effects against progressive joint destruction [6–8]. However, the clinical use of these therapies has been limited because of several issues, including side effects and the increase of therapy insensitive patients. Recently, several reports demonstrated that regulation of costimulatory signaling in the T cell is an important target for treatment of autoimmune diseases such as arthritis [9, 10]. CTLA-4 is an activation-induced

surface molecule on T cells and is essential for the negative regulation of T cell activation; its inhibitory effects can be accomplished either by competition with CD28 for their ligands B7-1 and -2 or by transmission of negative signals through its intracellular domain [11, 12]. The cytoplasmic domain of CTLA-4 (ctCTLA-4), which contains a tyrosine phosphorylation motif, has been found to be 100% conserved among different species, suggesting that this domain is critical for CTLA-4 functions [13, 14]. In addition, there are several splicing variants of CTLA-4, including full-length CTLA-4, soluble CTLA-4 that lacks the transmembrane domain, ligand-independent CTLA-4 (liCTLA-4) that lacks the extracellular domain, and only the cytoplasmic domain of CTLA-4 [15]. Data collected in a whole-genome association study usually consist of dense SNPs, with multiple SNPs covering a gene. In this paper, we use a similar gene-based analysis to study interactions between CTLA-4, IL-1 $\beta$ , IL-10. We used GC rich SNP's for polymer designing.

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## 2. Materials and Methods

The study was approved by Institute Ethical Committee and consent was obtained from all subjects before the study being started. Samples were collected from CHC Hospital, Kannauj, (U.P.) under the guidance of expert rheumatologist. 30 control (nor rheumatoid factor positive neither any arthritis patients) and 30 rheumatoid arthritis patients of both the sexes were taken. Venous blood was collected in EDTA vials. The samples collected have been processed to check the rheumatoid factor and DNA isolation from PNBs.

Rheumatoid factor was determined in all the samples by kit method (HUMATEX-RF). Only rheumatoid factor positive samples were preceded for further analysis.

### 2.1. Isolation of PBMCs from blood

For PBMCs isolation blood is taken into oaridge and 0.9% of NaCl is added for washing. Centrifugation is done at 6000rpm for 7 minutes at 4°C. Obtained dark pallet is further processed with solution A (sucrose 0.30M, MgCl 1 M, Triton X-100 1%v/v) and again centrifuged at 6000rpm for 7 minutes at 4°C. The obtained pellet is further rewashed by solution A. The pellet obtained is PBMCs pellet.

### 2.2. Isolation of DNA from PBMCs

The PBMCs pellet is solution B (Tris 1M, pH 8, EDTA 0.5 M, NaCl 1M, SDS 20%w/v) is mixed and inverted for 5 minutes. Now 5 M sodium per chlorate is added and incubated for 5 to 10 minutes. Centrifugation is done at 7000rpm for 10 minutes at room temperature by adding chloroform. Addition of 4ml isopropanol is done in aqueous phase for precipitation of DNA. Now thread like DNA is visualized and collected in ependorffs followed by washing with 70% ethanol. The DNA is left for drying and dissolved in 200 micro liter Tris- EDTA (Tris 1 M, EDTA 0.5 M).

### 2.3. DNA Validation

For validation of DNA run isolated DNA samples (2 micro liter DNA + sample + 2 micro liter gel loading due to phycol 15%, BPB 0.25%, cyanol 0.25%+ 6 micro liter MQ water on 0.8% agarose gel+ 2ml TAE (50X)+ EtBr 0.1 gm%).

### 2.4. Primer Designing

All the genes implicated in the pathogenesis of rheumatoid arthritis were identified with nucleotide sequence from NCBI. Two of them contained microsatellite motif so primers were designed for the upstream and downstream regions flanking microsatellite. The primers were designed using Primer -3 tools.

The primer sequence

#### IL1Beta

Left primer: GCCTGGACTTTCCTGTTGTC

Right primer: ACTTCTTGCCCCTTGAAT

#### IL-10

Left primer: TTCTCTCTTCTGGGACTGC

Right primer: TTCAGGTCTGGAAACGCTCT

#### CTLA4

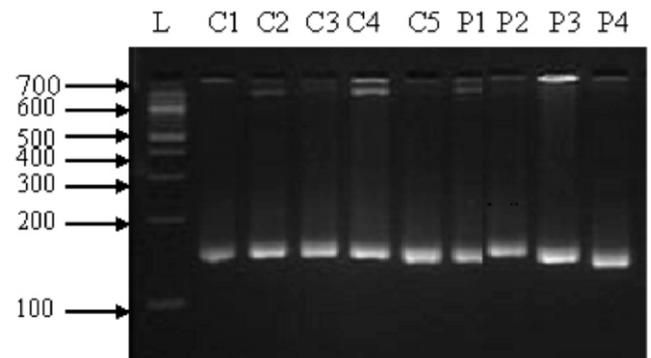
Left primer: CAGCCAGGAAGTGAAAGTCC

Right primer: ATTTCTCATCCCCACTGCTG

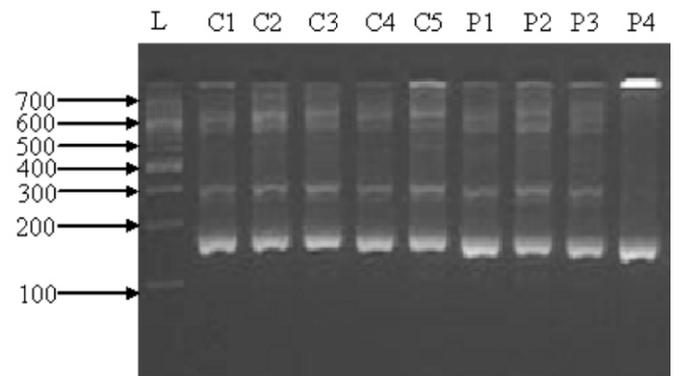
The PCR was standardized for different annealing temperature for different primers.

## 3.0. Results

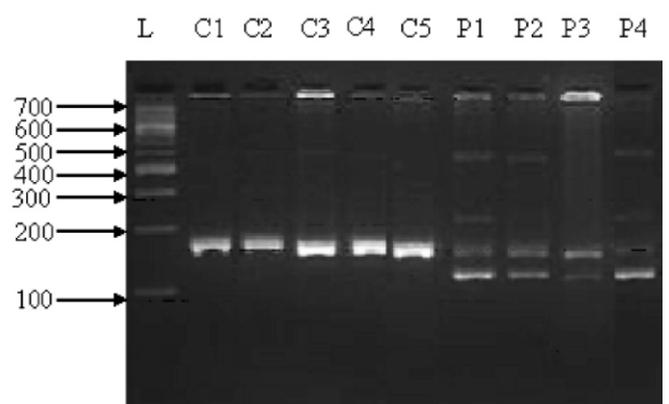
Patients were screened on the basis of radiological and clinical parameters and RA factor was estimated. All the patients were found to be RA positive clinically and rheumatoid arthritis patient.



**Figure 1.** shows the 6% PAGE of IL-1 $\beta$  gene in which no significant polymorphism is found between healthy controls and RA patients. Interleukin-1 $\beta$  plays an important role in the up- and down-regulation of the acute inflammatory response. It also plays a major role in a wide range of inflammatory and autoimmune diseases.



**Figure 2.** shows the 6% TAE PAGE of IL-10 gene. Like other cytokines interleukin-10 has many effects upon the functions of cells such as lymphocytes, monocytes, natural killer cells, and dendritic cells. Specifically, IL-10 is a cytokine that regulates immune-mediated inflammation. This figure shows no significant polymorphic pattern between controls and RA patients.



**Figure 3.** 6% polyacrylamide gel of CTLA-4 gene shows a polymorphic band at approximately 125 bp range in RA patients as compared to controls. Four polymorphism of the CTLA 4 gene have been identified in humans. There is a C→T transition at position -318 of the promoter sequence and a G→A transition at position +49 of exon-1, resulting in an alanine to threonine amino acid substitution in codon 17 of dinucleotide repeats of about 7-32 ATs in exon-3 and a fourth has been mapped to the 3' untranslated region of the CTLA 4 gene. All four polymorphism has been investigated for linkage with autoimmune disease.

### 3.1.Laboratory findings

No tests are specific for diagnosing RA. However, RF which is polyclonal immunoglobulin-M autoantibody reactive with the Fc portion of IgG, is found in more than 2/3 of adult with the disease. Widely utilized tests largely detect IgM RF. A negative RF dose not rule out RA, rather the arthritis is called seronegative. During first year of illness, RF is frequently negative. 80% of patients eventually convert to seropositive status. RF is also seen in other illness like SLE, Sjogrens syndrome, sarcoidosis, hepatitis B, leprosy, etc. and in approximately 10% of the healthy population, therefore the test is not very specific. Because of this low specificity, a new serological test has been developed in recent years. Which test for the presence of so called anti-citrullinated protein antibodies (ACPA).

### 4.Discussion

CTLA-4 is a costimulatory molecule for negative regulation of T cell activation. The fact that the amino acid sequences of the intracellular tail of CTLA-4 are 100% conserved among mammalian species suggests that signal transduction via this cytoplasmic tail has an important inhibitory role in T cell activation [12, 16]. The cytoplasmic portion of CTLA-4 is 36 aa in length, lacks any intrinsic enzymatic activity, and does not contain a bona fide ITIM motif; however, it contains many other potential protein-protein interacting motifs [15, 17, 18]. Allelic association between specific polymorphism of CTLA4 gene and an ever increasing number of autoimmune disease including RA [19]. Barton et al, 2000 [20] shows that the contribution of CTLA4 polymorphism to the risk of developing rheumatoid arthritis is still controversial. Whereas some studies shows that no association of the CTLA 4 polymorphism in people from Spain, UK and Korea [21, 22]. More detailed studies combining the CTLA 4 polymorphism with HLA genotype of patients found a correlation between the G-alleles of CTLA 4 (+49) and the HLA genotype HLA DRB1, known to be a susceptibility gene for RA [23, 24, 25]. This finding stress that the inheritance of autoimmune disease are most probably due to multiple susceptible genes. The four polymorphism of the CTLA 4 gene has been identified in humans. There is a C-T transition at position -318 of the promoter sequence and a G-A transition at position +49 of exon 1. A third polymorphism is a dinucleotide repeats of about 7-32 ATs in exon 3 and fourth have been mapped to the 3' untranslated region of the CTLA 4 gene. B.Vaidya et al, 2002 [26] shows in his study that the G allele of the CTLA 4 A/G polymorphism is more prevalent in early RA patients than in control.

IL 1 $\beta$ , a cytokines produced by monocytes mediates cartilage and bone destruction in RA. IL 1 $\alpha$ , IL 1 $\beta$  and their naturally

occurring antagonist, IL 1Ra are encoded by genes located within a 430 kb region. IL 1 Ra contracts the action of IL 1 by binding to IL 1 receptor without activation it [27].

In many studies the IL 1 $\beta$  polymorphism was listed in control and rheumatoid patients. However we could not find any kind of allelic variation in the patients and control but studies suggest that IL 1 $\beta$  polymorphism is associated with the development of rheumatoid arthritis. But my study was conducted on a smaller group of patients. Larger group would be suggestive of involvement of IL 1 $\beta$ , association with rheumatoid arthritis in the Indian population. IL 10 is suppresses pro-inflammatory cytokines and chemokins derivatives macrophages and inhibits T cell proliferation [28]. In view of these previous observations, the finding of [29] studies support the notion that IL 10 rises in response to inflammation as an attempt to keep it in check out falls short of mitigating joint inflammation [30] It has also been suggested that production of IL 10 occurs relatively late in the immune process; after cell activation and pro-inflammatory cytokines release, there by pointing towards role of IL 10 as a natural dampener of the immune process [31]. It is important to note that IL 10 not only down regulates but also up regulates a number of immune functions such as enhancement of Fc receptor expression on monocytes/macrophages, stimulation of IgG mediated phagocytosis, endocytosis and antibody-dependent cell mediated cytotoxicity. In my observation the IL 10 polymorphism was observed in control and rheumatoid arthritis patients. But we could not find any polymorphic variations in patients and control, but studies suggest that IL 10 polymorphism and production associated with limitation and termination of inflammatory responses and the regulation of differentiation and proliferation of several immune cells such as T cells, B cells, NK cells, antigen presenting cells and mast cells and granulocytes. However recent data suggest that IL 10 also mediates immunostimulatory properties that help to eliminate infectious and noninfectious patients with limited inflammation. But in my observation, we could not find any significant association between control and RA patients for involvement of IL-10.

### 5.Conclusion

When we tested for its association in Indian RA patients and control is showed polymorphism of CTLA-4 in selected patients. We cant find polymorphism of IL-10 and IL-1 $\beta$ . However for its association with the disease the study needs to be performed on a larger group of patients.

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