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Original article

Evolving clinical profile of HLA-DRB1, MMP1 and NF-κB gene in rheumatoid factor positive Caucasian population 1.

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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 NFkB1 and MMP-1. However HLA-DRB1 showed polymorphism in RA and controls. 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).

1. Introduction

Rheumatoid arthritis (RA) is a symmetric, chronic polyarticular arthritis that effects 0.5 – 1 % of world population. The incidence of RA is more than six times as great in 60 to 64 years old women compared to 18-19 years old women [1]. It primarily causes progressive joint destruction that leads to restriction of daily activities and deterioration of quality of life. Etiopathology of RA is not fully known, it's thought to be a disease caused by combination of multiple genetic and environmental factors. It's a common systemic inflammatory autoimmune disease. RA is characterized by symmetrical joint involvement can cause irreversible joint deformities and functional impairment. This disease appears three times greater in woman (Matsumoto AK, Johns Hopkins). Its onset is usually early in women; commonly begin in child bearing age [2]. Previous studies have indicated few genetic regions that might be associated with RA most notable HLA- DRB1. The HLA region on 6p21 is well known for showing the strongest association with RA. The evidence so far has particularly pointed to HLA-DRB1 gene [3]. The PTPN22 gene (1p13.3-p13.1) has also repeatedly shown association to RA, in a modest way. Despite these findings, progress in identifying new genes associated with the susceptibility to RA has been limited. 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 HLA-DRB1, MMP-1, Nf-κB. We used GC rich SNP's for polymer designing.

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 PNBCs.

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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 6000 rpm 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 6000 rpm 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 7000 rpm for 10 minutes at room temperature by adding chloroform. Addition of 4ml isopropenol 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

1. HLA-DRB1 (MHC-II)

Left primer: GGGACAGGGCTGTTCATCTA
Right primer: CTTCAGGCCAAGAACAGGAG

2. NF-κB1

Left primer: AGAGAGGGAAGGTGGTTGGT
Right primer: GGTGGCACTGTCCAAGAGTT

3.MMP1

Left primer: CATCCAAACTTGTCTCCACAAA
Right primer: TTTTCAACTTGCCTCCCATG

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

3.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 presence of isolated DNA when run on 0.8% Agarose gel on which isolated DNA was checked for their quantity. The entire DNA quantities from 2 µl falls under the range of 150-200 bp from this 25 µl DNA was preceded for PCR.

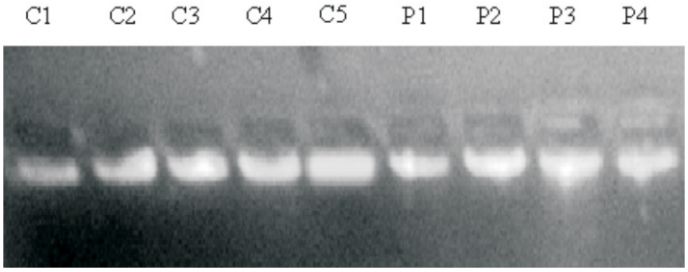

Figure 2. Shows the 6% TAE PAGE of MMP1 gene. In this observation MMP1 gene dose not show the any polymorphic association in a study between control and RA patients. In RA, the MMPs are locally produced and activated within the affected joint as a result of cytokine mediated stimulation of synovial cells.

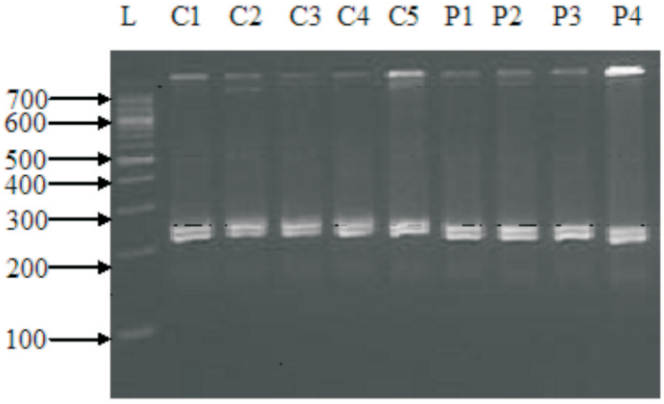

Figure 3. Shows 6% TAE PAGE of NFkB gene, which does not, shows any significant polymorphic association between healthy individual and RA patients. NF-kB is a transcription regulator that is activated by various intra and extra-cellular stimuli such as cytokines, oxidant free radicals, and viral products. Inappropriate activation of NF--kB has been associated with a number of inflammatory diseases, while persistent inhibition of NF-kB leads to inappropriate immune cell development or delayed cell growth.

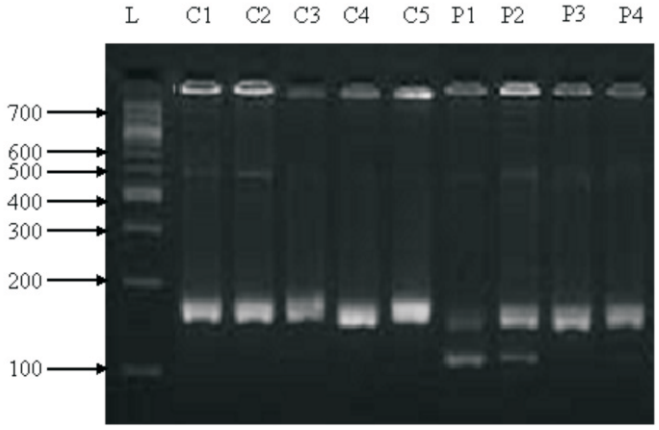

Figure 4. Shows 6% TAE PAGE of HLA DRB1 gene shows a polymorphic band at approximately 125 bp range in RA patients as compared to control. In this observation only two patients P1 and P2 shows polymorphic association but no significant association was found in rest of two patients P3 & P4. Hence more number of patients is needed to validate the polymorphic association of HLA-DRB1 gene with RA.

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

Rheumatoid arthritis is traditionally considered a chronic multisystem, inflammatory autoimmune disorder that causes the immune system to attack the joints. It is a disabling and painful inflammatory condition, which can lead to substantial loss of mobility due to pain and joint destruction. Rheumatoid arthritis is a systemic disease often affecting extra articular tissue throughout the body including the skin, blood vessels, heart, lungs and muscles. Cytokines are key player in pathogenesis of synovitis and subsequent joint damage in rheumatoid arthritis. Difference in levels of various cytokines among different individual can be possible explanation for difference in disease susceptibility and severity.

In our study, when tested for its association in Indian RA patients and control showed polymorphism in 3/2 of the five patients selected. However for its association with the disease the study need to be performed on a larger group of patients and control and its association with other disease like idiopathic juvenile arthritis and AS has to be carried out. The destruction of cartilage and bone in RA is mediated by proteolytic enzymes secreted by an inflammatory synovial tissue [4]. Because destructive enzyme of the matrix metalloproteinase (MMP) family are involved in this possibly influenced the courses of RA. The genes of MMP 1 are located at the long arm of chromosome 11 at position 11q22 (Spurr NK et al, 1988). In recent years, functional relevant polymorphism of these enzymes has been detected. The promoter region of MMP 1 contains a guanine insertion/deletion polymorphism (1G/2G) at position -1607 [5]. The 2G allele results in increased transcriptional activity because the guanine insertion creates a binding site for a member of the ETS transcription factor family [6]. The 2G allele may contribute to increased invasiveness of colorectal tumors [7] to the development of cancer [8] and lung cancer [9] In the present prospective follow up study of early RA patients, the significance of MMP 1 promoter polymorphism in relation to disease activity and radiological damage. MMP 1 together with MMP 13 play dominant role in the progression of RA, making prime targets for arthritis therapies. Since both the enzymes are transcriptionally activated by inflammatory cytokines [10].

Similarly other recent investigations like the study of Salvia et al, [11] failed to detect any correlation of the MMP 1 polymorphism with the susceptibility to RA. This is not surprising, given the widely accepted perception of RA as a disease that is dependent on, if not initiated by T cell driven antigen-dependent mechanisms, labeling tissue destructive process as a secondary phenomenon. However, functional relevant allelic polymorphism of MMP 1 genes, specifically the MMP 1 polymorphism, could influence the severity of the disease. The 2G allele of MMP 1 is associated with a higher promoter activity in vitro [12].This leads to the production of increased amounts of MMP 1 protein [13]. No association seen between MMP 1 polymorphism and disease severity. In promoter polymorphism were not associated with radiological damage or progression after a follow up of two years.

According to our observation, there was no MMP 1 gene polymorphism was listed in control and RA patients. However we could not find any kind of allelic variations in the patients and control. But studies suggest that MMP 1 promoter polymorphism associated with the development of RA. The 2G allele of MMP 1 is associated with a higher promoter activity in vitro, which leads to the production of increased amounts of MMP 1 protein. Because our study was conducted on smaller group of patients, so for the significant result larger population would be required for the association of MMP 1 gene with RA in the Indian population near and across Kanpur city. NF-kB gene encoding a 105 KD protein which can undergo constastional processing by the 26S proteosome to produce a 50 KD protein. NFkB is a transcription regulator that is activated by various intra and extra-cellular stimuli such as cytokines, oxidant free radicals, ultraviolet irradiation and viral products. Inappropriate activation of NFkB has been associated with a number of inflammatory diseases.

Ninomiya-Tsuji et al [14] in 1999 investigate that NF-kB activates the signaling pathway when it is induced by cytokines. Upon binding of IL 10 to its cognate receptor, transforming growth-factor-β-activated kinase becomes active, leading to the activation of the NF-kB inducing kinases (NIK). NIK responsible for the phosphorylation and activation of the inhibitor of kB (IKB) kinases, which then phosphorylate IKB . A loss of IKB leaves the dimer free to translocation to the nucleus and activates several genes inducing those for some MMPs. Bondeson et al [15] investigate in his study that activation of MMP 1 required NF-kB. This pathway is another potential therapeutic target, in which over expression of IkBα reduced expression of inflammatory cytokines and MMPs, but did not reduce anti-inflammatory cytokines or TIMP. Furthermore, mice deficient in the p50 subunit are refractory to collagen induced arthritis [16], indicating that the factor has a prominent role in the arthritic disease.

In our study the NF-kB polymorphism was listed in control and rheumatoid patients. But we did not find any kind of allelic variation in the patients and control but studies suggest that polymorphism in NF-kB is associated with the development

of RA by activation of MMPs and other susceptible genes. Our study was conducted on a smaller group of patients and we could not correlate the association of NF-kB with RA in Indian population.

The mechanism of the association between the HLA-DRB1 component and RA remain currently uncertain. The presentation ability of at risk alleles is likely related to their amino acid sequence but could also be influenced by characteristics of their expression. It has been shown that HLA-DR membrane expression was directly related to DRB1 transcript amount [17]. Allele dependent variation of HLA heterodimers expression on B cells with functional consequences on T cell immune response has been specifically shown in RA patients. In this view, whatever the mechanism of DRB1 deregulations is primary or induced by cytokines for example, variation in the expression of DR molecule on B cells could have important consequences on immune response, possibly leading to autoimmune process.

When we tested for its association in Indian RA patients and control is showed polymorphism in 3/2 of the five patients selected. However for its association with the disease the study needs to be performed on a larger group of patients and control and its association with other disease like idiopathic juvenile arthritis and AS was to be carried out.

5. Conclusion

Our study was aimed to find any disease correlation with respect to a few genetic loci implicated in rheumatoid arthritis. So studies were conducted for Human leukocyte antigen (HLA-DRB1), Nuclear factor of Kappa B1 (NFkB1), Matrix metalloproteinase-1 (MMP-1). No significant association was observed for NFkB1 and MMP-1. However HLA-DRB1 showed polymorphism in RA and controls. Therefore the studies need to the conducted on larger group of patients, so that the association can be verified in Indian population.

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