International Journal of Genetics, ISSN: 0975–2862, Volume 1, Issue 2, 2009, pp-06-09

Analysis of the genetic diversity of arthritis patients with reference to the factors inducing its onset

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Abstract- A lot of research on the prevalence of arthritis in India population has been carried out. However no studies on the genetic relationships within groups of arthritic patients in India have been reported. The current study focuses on analyzing the genetic diversity between patients suffering from the various types of arthritis with reference to factors inducing its onset. A total of 36 patients suffering from different types of arthritis were analyzed with reference to the age, gender, BMI, family history, hemoglobin and thyroid profile. The variation in the genetic profile was also analyzed using the RAPD technique. Data analysis showed patients suffering from arthritis to be at a greater risk of thyroid disorders, however the type of arthritis played a significant role. A distinct correlation between the genetic variation and presence of arthritis including its type was observed.
Key words **-** RAPD, Arthritis, Seronegative

Introduction

The term Arthritis is used to describe changes in the joints which may be either inflammatory or degenerative in character. If only one joint is affected the condition is referred to as monoarticular arthritis; if several joints are involved it is called polyarticular arthritis or polyarthritis. Based on the different causes, arthritis are of different types. Arthritis is one of the oldest diseases in the world as we see in history. According to a Finnish estimate, the incidence of enteroarthritis or uroarthritis per 1 lakh adults is 30 whereas that of seronegative arthritis is 40 per year. (10) Rheumatic fever, often occurs in developing countries but is rare in developed countries. The maximum prevalence of Arthritis is in Indians followed by Americans [1-5]. The prevalence of rheumatoid arthritis in India is about 5% out of the total Indian population of 1,065,070,607 [19]. There are many factors which may causes arthritis including environmental factors, genetic factors, as well as non genetic host factors. In the present study we have analyzed the genetic variation of patients suffering from the different types of arthritis along with an analysis of their age, gender, BMI, family history, hemoglobin and thyroid profile. A correlation between the type of arthritis and genetic profile was done.

Material and method

Selection of patients and collection of data

Blood collection is done from different institute and laboratory of Gujarat [11-14]. A total of 36 patients, including 27 women, aged between 23-64 years (median = 43), 3 children and 6 men presenting arthritis were evaluated. These included patients suffering from both rheumatoid arthritis and osteo arthritis. Patients identified as suffering from rheumatoid arthritis were classified as sero-negative and sero positive according to the results of the autoantibody detection with the doctors' guidance. A data of their age, gender, BMI, family history, hemoglobin and thyroid profile was generated.

RAPD Analysis

Blood D.N.A. isolation

Three ml of blood was centrifuged at 2000 rpm at 4ºC for 10 mins and the plasma layer discarded. To the pellet 2ml of lysis buffer was added and incubated for 30 min at -20ºC. The sample was than centrifuged at 3000 rpm for 10 mins and the supernatant discarded. The pellet was again washed with 2ml of lysis buffer followed by centrifugation at 3000 rpm for 10 mins. The supernatant was discarded and the washing step repeated till the red color was removed. This was followed by addition of 0.5 ml of S.E. buffer to the pellet along with 0.25ml of Proteinase K. Finally the pellet was incubated in the water bath at 50ºC overnight. The suspension was extracted with equal volume of phenol: chloroform (24:1) solution. This was centrifuged at 10,000rpm for 5 min. The upper (aqueous) phase was transferred into a new eppendroff tube. The supernatant was extracted with an equal volume of phenol: chloroform: iso amyl alcohol (25:24:1) and centrifuged for 5 min at 10,000rpm. The upper (aqueous) phase was again transferred into a new eppendroff tube mixed with 1/3 volume of sodium acetate and washed with double volume of 70% Ethanol (-20 deg C). This was incubated at -20ºC for 20-30 minutes and centrifuged at 10,000rpm for 15-30 min at 4ºC. The pellet was allowed to air dry for 15 mins and resuspended in 50-60uL TE buffer. The obtained DNA samples were subjected to RNase treatment and the qualitative and quantitative analysis were carried out using 0.8% agarose gel electrophoresis and spectrophotometer measurement (Unicam Alpha) at 260 nm. The obtained DNA samples were stored at -20ºC until further use [15-19].

PCR

Fourteen samples showing good DNA isolation were selected for the RAPD analysis. PCR reactions were carried out in 12.5 µl volumes in which 100 ng of target DNA after standardization for best results was used. Amplifications were performed using the following reaction mixture: : 0.3 µM of primer, 200 µM of dNTPs, 0.75 units of Taq DNA polymerase diluted appropriately with 10x assay buffer containing 15 mM $MgCl_2$ (Bangalore Genei, Bangalore) The temperature profile for all the reactions was 95ºC for 5 minutes followed by 40 cycles of 95ºC for 30 s, 32ºC for 1 min, 70ºC for 1min with a final extension at 72ºC for 5 minutes. Amplification products were maintained at 4ºC until electrophoresis. The reaction products were analyzed by loading the total amplified product in1.5 % agarose gels containing ethidium bromide (11). Electrophoresis was performed at 100V for approx. 3h. Five 10-mer primers were selected for the present study as listed in Table 1.

Analysis of data

Only well-resolved fragments observed in the gels were scored. Gels were scored independently by at least two individuals Fragments that migrated to the same position on these high-resolution gels were considered to be from homologous loci. A similarity matrix was created using Jaccard's similarity coefficient (16), which does not consider joint absence of a marker as an indication of similarity. Clustering was done using unweighted pair group arithmetic mean method (UPGMA) .The final dendrogram was created using a combination of two programmers, Free tree and Tree Explorer (11).

Table 1- List of primers used for RAPD study

Primer Number	Sequence
1	ACAACGCCTC
2	GGGAACGTGT
3	CTGGGCAACT
4	CCTTGACTCA
5	GGGACGTCTC

Results

Selection of patients and collection of data

Data analysis of samples with respect to different factors affecting arthritis was done as shown in table 2. Forty percent of patients were found to be suffering rheumatoid arthritis, 25% form osteo arthritis were as 30% were sero negative (Figure 1). The collected samples of arthritis affected patients showed a male female ratio of 1:3, where 70% of the patients were aged between of 41-60. This age showed maximum prevalence of arthritis. The study of the BMI ratio, showed that 65% of the patients were over weight or fat with no patients showing low BMI ratio (corresponding to thin body built). When focusing on the thyroid profile, 60% of the R.A. patients were thyroid positive where as only 40 % of were thyroid positive in case of the O.A. patients. Of the sero negative cases only 30% were found to be thyroid positive. Another clinical factor known to be associated with arthritis is the hemoglobin range. When analyzing the hemoglobin profile of the arthritis affected patients 70% were found to have low range of hemoglobin. The family history of arthritis was shown maximally in cases of osteo arthritis with about 60% showing positive family history, followed by 35% in sero negative patients and only 15 % in R.A. patients.

Table 2- Data analysis of samples with respect to different factors affecting arthritis

Type of Arthritis à	Rheumatod Arthritis	Osteo-Arthritis	Sero	Total
			Negative	
Patients	16	9	11	36
Gender				
Male	4	2	3	9
Female	12	7	8	27
Age				
0-10 year	0	0	0	0
11-20 year	1	1	2	4
21-30 year	1	0	1	2
31-40 year	3	0	0	3
41-50 year	4	4	3	11
51-60 year	5	4	5	14
61-70 year	2	0	0	2
BMI Ratio				
Thin(>20)	0	0	1	1
Normal(20-25)	6	2	4	12
Fat (<25)	10	7	6	23
Thyroid				
Positive	9	4	3	16
Negative	7	5	8	20
Hemoglobin				
Low(>12gm).	12	7	6	25
Adequate(12-18gm)	4	2	5	11
Family history				
History	2	6	4	12
No history	14	3	7	24

RAPD Analysis

Blood DNA isolation

The DNA extracted from the blood samples was run on a 0.8% agarose gel and 14 samples showing high quality of DNA were selected for further analysis.

R.A.P.D.

The obtained data for the genetic diversity of the arthritic patients showed two main clades. One in

which all the rheumatoid arthritic patients were grouped together where as the other in which all the other samples were seen. In the second clade, Clade B, there were two main groups. Group 1 showed the presence of all the sero negative and normal samples and Group 2 showed the presence of the samples from patients suffering from osteo arthritis. Figure 2 shows the Agarose gel showing the RAPD profile of the samples using primer 2. The dendogram generated showing the genetic diversity of all the samples is shown in Figure 4.

Conclusion

The current study supports the reports that arthritis is 2-3 times more common in females than in males. It emphasizes that the frequency of arthritis increases with age and peaks in persons aged above 50 years. Cases of arthritis are mostly observed in obese or over weight people. The study confirms the theory emphasizing that there may be a common genetic link between RA and autoimmune thyroid disease; however the type of arthritis also plays a significant role in determining the presence of thyroid disorders. It can be concluded that there is increased genetic variation in patients with arthritis. However, there is a correlation between amount of variation and the type of arthritis. The study also suggests that the sero negative patients have relatively similar genetic profile to the normal samples as compared to the patients suffering from osteo arthritis. However the genetic profile of all rheumatoid arthritis patients is distinctly different. A detailed analysis of the genetic variation in large populations could prove vital in developing a tool for diagnosis of the different types of arthritis in Indian population.

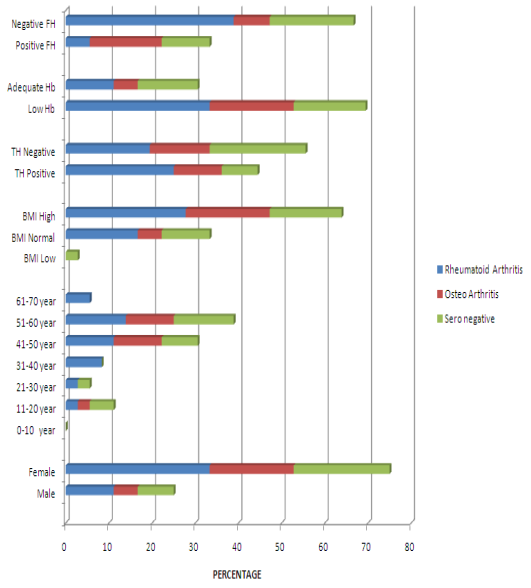

Fig. 1- Data shows in bar chart

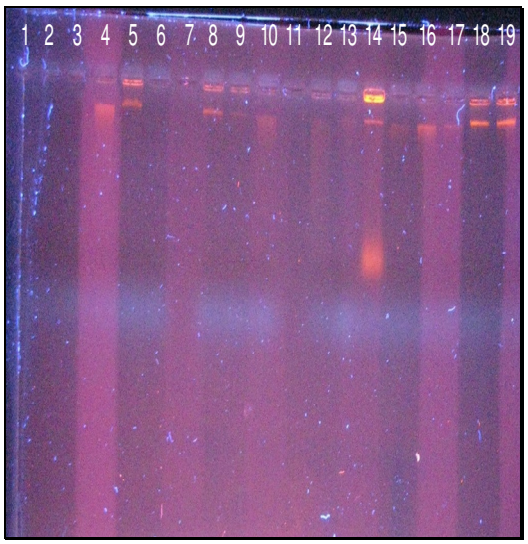

Fig. 2- Agarose Gel showing the quality of DNA

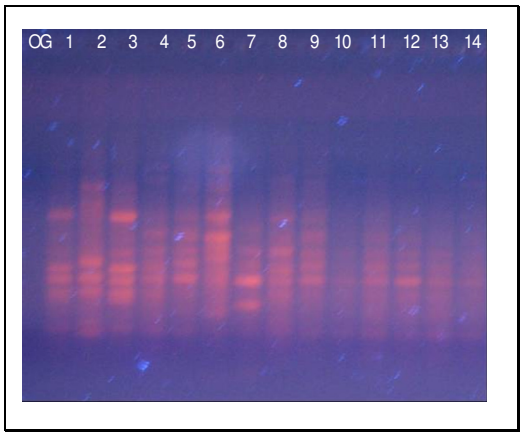

Fig. 3- The agarose gel showing the RAPD profile of the samples using primer 2

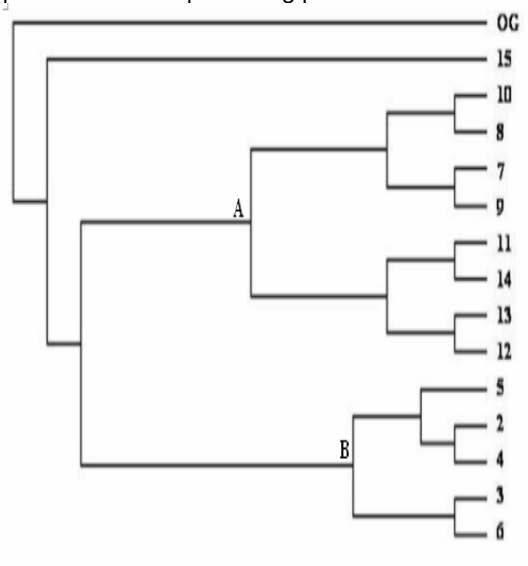

Fig. 4- The dendogram generated from the RAPD analysis of the 14 samples used in the study

Acknowledgement
Special thanks to Dr. M. N. Prabhkarn, Dr. Lira Mahapatra, Dr. Bhavin Bhatt, Dr. Viradia, Dr. Sanjiv Ravisaheb, Mr. Amit Trivedi, Mr. Vishal, Mr. Hiren Oza and Mr. Nirav Vyas, Aditi Pathology Laboratory, Krupa Pathology and Sir T. Hospital Bhavnagar for their support.

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