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Studying the Effect of Genetic Diversity in Samples of Iraqi Arthritis Patients on Response to Drug Treatment Using RAPD/PCR Technology

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ABSTRACT

Rheumatoid arthritis (RA) is an autoimmune disorder of unknown etiology, despite therapy may result in progressive joint destruction, deformity that lead to disability, and even premature death in some cases. Random Amplified DNA Polymorphism (RAPD) technology showed high efficiency in investigating the genetic diversity between healthy control group and RA patients and detecting the genetic diversity among RA patients and the effect of this diversity on the patients response to the drugs regimens used to treat RA. The primers GATGACCGC and ACAACGCCTC showed the best ability to discrimate between the healthy subjects and the RA patients, and the primers GATGACCGC, ACAACGCCTC and CAGCACCCAC showed the best ability to detect the genetic diversity among RA patients and detecting the effect of this diversity on the patients' response to treatments. Analysis of the resulted pattern of amplifications through the Unweighted Pair Group Method with Arithmetic mean (UPGMA) algorithm program revealed an important thing which is the fact that RAPD technology is an important relatively cheap and available technology that can be used in pharmacogenomics because of the fact that there is an association between the genetic content of the patients and their response to different treatment regimen.

Key words: RAPD/ PCR technology, rheumatoid arthritis genetics, population genetics, Iraqi population genetics

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INTRODUCTION

Rheumatoid arthritis (RA) is a complex, systemic, chronic inflammatory with autoimmune features which is associated with significant disability and early mortality, it affects approximately 1% of the population worldwide and is therefore one of the most common autoimmune diseases [1,2]. RA is not a rare in Iraq it have been previously demonstrated in Iraqi population with more frequency in women than men [3,4,5].

There are different regimens used to treat RA including traditional non biological treatments and the recently used biological treatment [6,7]. Nonsteroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids are first line therapies used for symptomatic relief of RA, in conjunction with the use of NSAIDs, disease-modifying antirheumatic drugs (DMARDs) are promptly initiated in the management of RA, DMARDs attempt to slow down the progression of RA and include: methotrexate (MTX), sulfasalazine, and hydroxychloroquine. These agents are either used as monotherapy, or more commonly as part of a cocktail with multiple DMARDs or other biologycal agents [8,9].

Although the exact etiology of RA is unknown, the genetic predisposition in combination with environmental triggers contributed to increase the risk for rheumatoid arthritis[10]. Previous studies have shown that genetic factors influence the response to drugs used in RA therapy; There are many reasons for such interindividual variability, but an increasing attention has been focused on the genetic component, in a scientific field known as Pharmacogenetics and pharmacogenomics[11].

For the mentioned reasons and the absence of any pharmacogenomic studies in Iraq or any other studies about the factors that controls or predict the patients response to the different drug regimens used in the treatment of RA, this study is planned to detect the role of individual genetic differences of the patients and their impact on the patients response to treatment by studying the effect of genetic diversity using random amplified DNA polymorphism (RAPD) technology on a sample of Iraqi RA patients responding to traditional treatment.

MATERIALS AND METHODS

The study was carried out from October 2012 till May 2013 on 84 subjects divided into two groups. The first group is the patients croup contained 56 Iraqi rheumatic arthritis patient selected from RA patients attended the consulting clinic of Mergan Hospital in Babylon city, who have previously diagnosed with RA for at least three months. Twenty eight patients of them taking MTX as treatment and the rest of them take corticosteroids for RA treatment.

The second group is the control group composed from 28 subjects of apparently healthy individuals from different Iraqi populations were selected randomly with no RA symptoms and had no family history of RA.

The exclusion of subjects from the control group was done by examining the serum of each subject with RF latex kit (Sinreact, Spain) to detect the subjects with the positive RA. Both the patients and the healthy subjects groups were asked different questions including the following: (age, gender, height, weight, presence of a family history with RA, presence of other autoimmune diseases and the presence of any other genetic diseases, and type of treatment they use). The study protocol was approved by the Ethics and Research Committees of the hospital, and all patients gave informed consent to the study.

DNA from whole blood was extracted using FlexiGene DNA Kit (Qiagen, Germany), DNA concentration and purity of the DNA samples were estimated by using nanodrop device (Fisher, USA). Then DNA stored at –20°C until used.

DNA finger printing and detecting the genetic diversity was done through RAPD analysis using the primers **GATGACCGCC**, ACAACGCCTC, CTGGGCAACT, CCTTGACTCA and CAGCACCCAC to generate amplicons under 2000 bp.

RAPD/PCR reactions for the both RA pateints and the healthy control group were carried out using GO Taq Green mastermix (Promega, USA), with a final reaction volume 25 µl containing: 1.5 µl primer, 3 µl DNA, 2X GO Tag Green Mastermix 12.5 µl and Nuclease free distil water 8 µl. Optimization of **RAPD/PCR** reactions was accomplished after several attempts to detect the best condition for the amplification by using Verti96 Thermo cycler (Applied biosystem, USA). After optimization the programs used to amplify each primer are mentioned bellow:

The genetic variations between subjects were detected by noticing the resulting RAPD patterns after electrophoresis on agarose gel 1.5% containing ethidium bromide (Promega, USA) under electrical power (50 volt) for 3 hours using *AgaroPower*™ device (Bioneer, Korea) and photographed using digital camera (Sony, Japan). The analysis of the genetic diversity, discrete amplified fragments across the lanes were scored. Every scorable band was considered as single allele/locus and was scored as present (1) or absent (0). Genetic similarity was calculated using Unweighted Pair Group of Arithmetic Means (UPGMA) program and the resulted similarity matrixes were used to draw the phylogenic tree for each primer using the same program.

RESULTS AND DISUCSSION

The primer GATGACCGCC gave 13 bands. Four of these bands were presented in all the tested subjects while the others where variable from group to group or variable within the same group. The patterns of PCR product arranged in 11 different patterns (Figure 1), some of these patterns in lane 2,3,4 and lane 8,9,10,11 and lane 12 found in higher frequency than the others while the pattern is found in lane 2 only once.

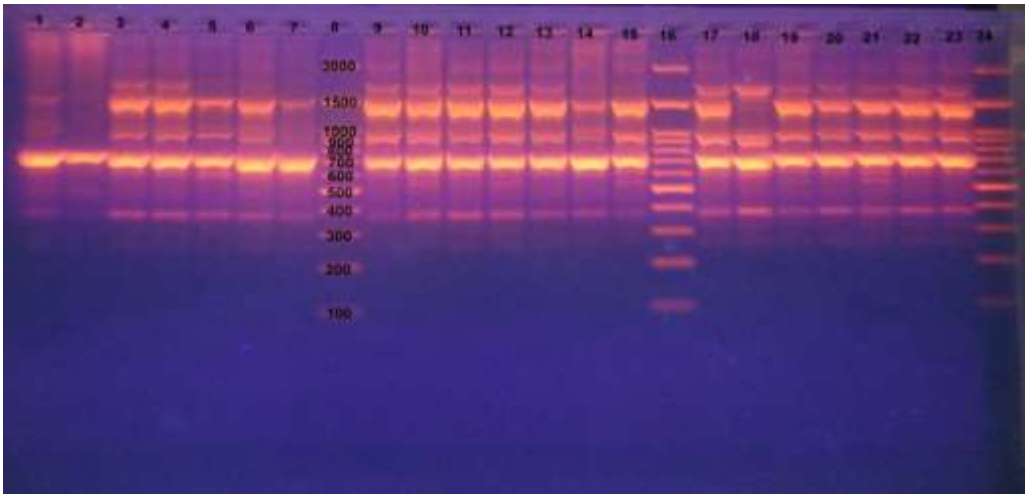

Figure 1: **RAPD Pattern for Primer GATGACCGCC on 1.5% Agarose Gel at 50 Voltages for 3 Hours**

Lane 1-7 RAPD patterns of healthy control subjects (H group) ; Lane 8, 16, and 24 H3 100 bp DNA Ladder ; Lane 9-15 RAPD patterns of patients using MTX as treatment (M group) ; Lane 17-23 RAPD patterns of patients using CS as treatment (C group)

To detect the genetic diversity among H, M and C groups the resulted RAPD patterns were translated into digital patterns of zeros and ones (zero=absence of band and one = presence of a band) and used to built phylogenetic tree of this primer figure 2 and in the resulted tree we noticed the following:

1. There was a big similarity between the H group and the subject in this group fell in 5 branches. Four of them were very much close to each other (much similar) and only one branch (H2) was far away from all the studied subjects. This means that the H group is much similar to themselves and genetically different from the other groups.
2. The M group fell in 4 braches. One of these branches repeated only once and was relatively close to the healthy group, the second branch (the most frequent pattern) arranged in a distant branch and two small branches have a kind of similarity with the C group and these 2 patterns repeated in small number of patients.
3. The C group arranged in 4 branches. One of them was quite distant from the other branches and its pattern repeated most frequently among patients reserving corticosteroid treatment and 2 branches (patterns) were quite similar to the M group and one branch (pattern) was repeated only once.

Primer **ACAACGCCTC** gave 14 band. Five of these bands were presented in all the tested subjects while the other nine bands were variable from group to group or variable within the same group the patterns of PCR product was arranged in 7 different patterns Figures (3) and (4).

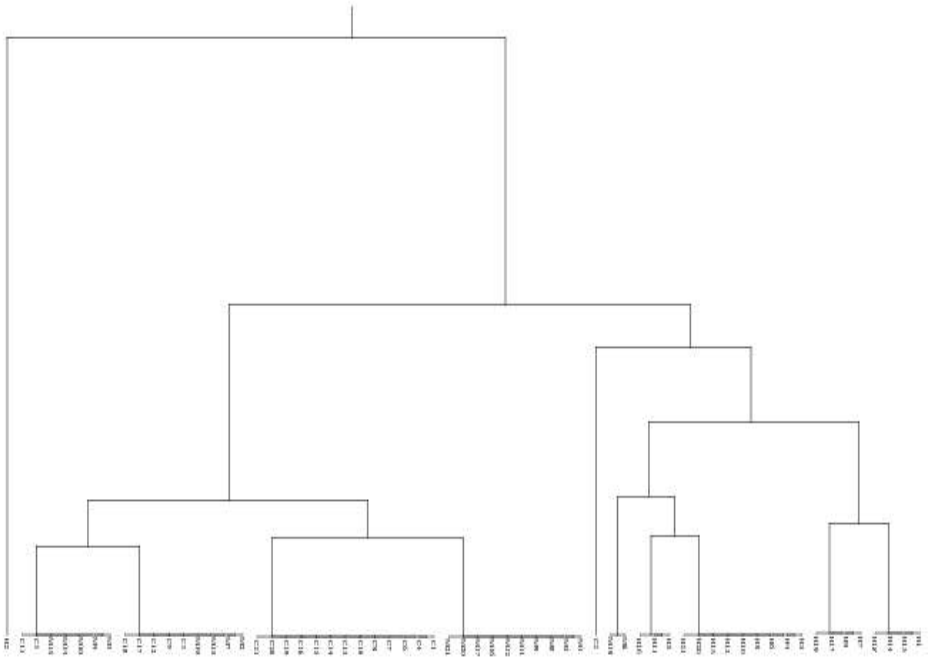

Figure 2: Phylogenetic Tree of Primer GATGACCGC Clustering Drawn the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) Algorithm Program

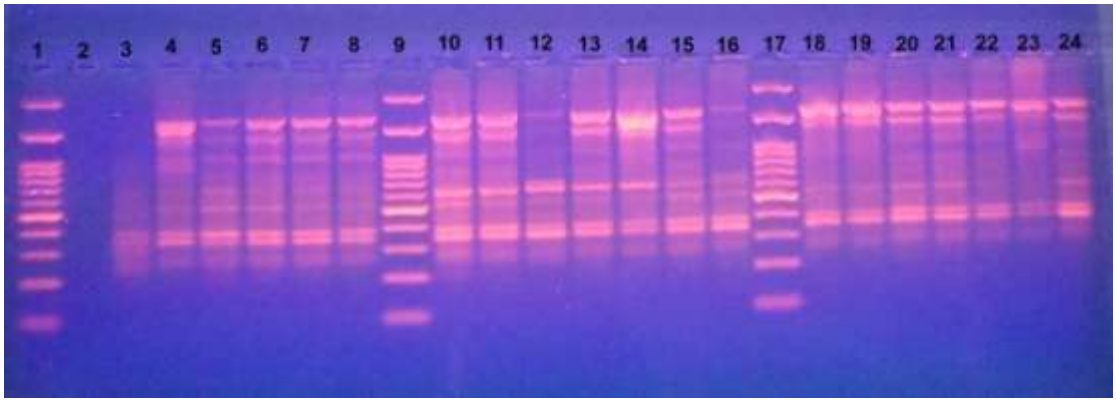

Figure 3: **RAPD Pattern for Primer ACAACGCCTC on 1.5% Agarose Gel at 50 Voltages for 3 Hours**

Lane 1, 9 and 17 H3 100 bp; Lane 3-8 RAPD patterns of healthy control subjects (H group) ; Lane 10-16 RAPD patterns of patients using MTX as treatment (M group) ; Lane 18-24 RAPD patterns of patients using CS as treatment (C group)

The phylogenic tree of this primer revealed the following:

1. The H group fell in 3 main clusters, the first cluster was similar to the pattern H1, the second pattern was similar to H3 and the third cluster was similar to H8.

The H1 and H3 patterns repeated most frequently while the H8 repeated only twice.

2. The M group also falls in 3 main clusters, M2 pattern in them was the most frequent pattern while M3 pattern is repeated only 4 times and M1 is repeated only once.

3. The C group fell in 5 clusters, the C1 pattern was the most frequent pattern followed by C2 pattern while pattern C13, C20 and C19 repeated only once, and the pattern C13 and C20 show the biggest similarity.

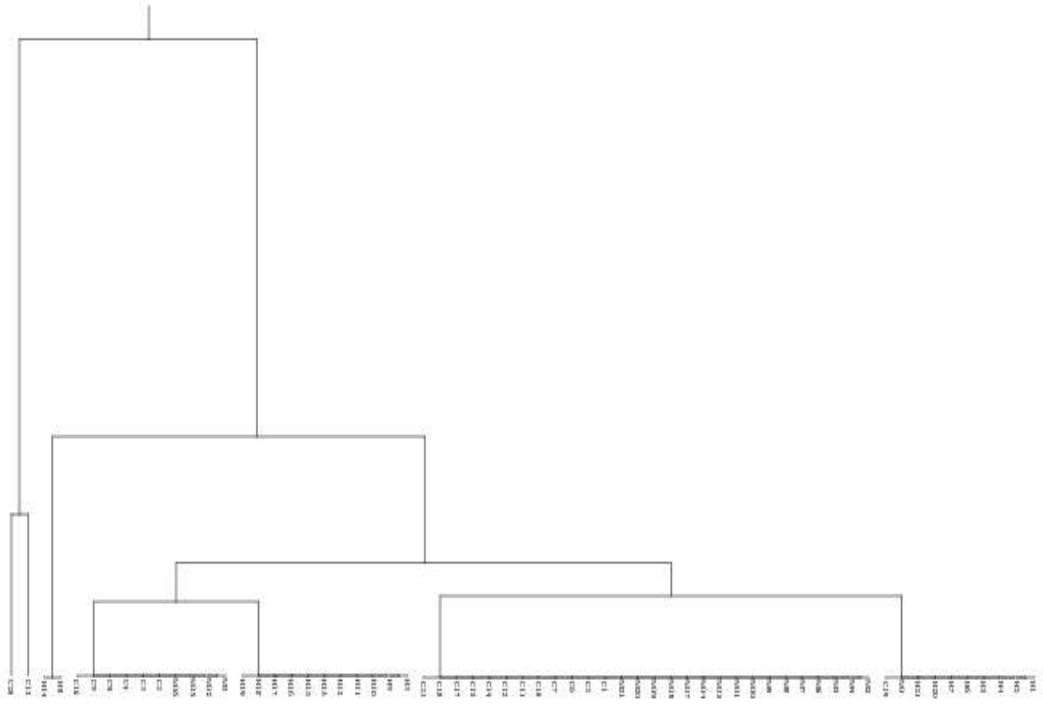

Figure 4: Phylogenetic Tree of Primer ACAACGCCTC Clustering Drawn the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) Algorithm Program

Primer CTGGGCAACT gave 7 bands all of them were present in all the tested subjects except 6 subjects who showed no PCR amplification for this primer figure (5).

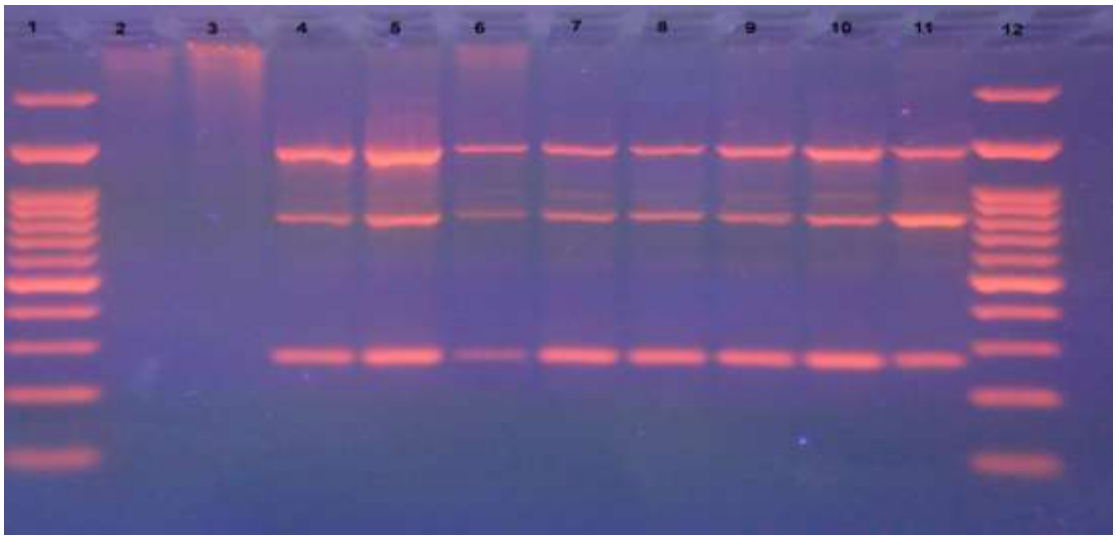

Figure 5: RAPD Pattern for Primer CTGGGCAACT on 1.5% Agarose Gel at 50 Voltages for 3 Hours

This means that the primer is not good for studying the effect of genetic diversity in RA patients because it gave a single pattern repeated in all the subjects included in the present study, but these results cannot assure that this primer is unuseful genetic marker to detect RAPD polymorphism among RA Iraqi patients because all the subjects included in this study were from the same geographical area (Babylon city) and only few subjects from other areas (Baghdad) and this geographic effect may have led to similarity in the numbers of receptors to this primer which lead to similarity in the resulted RAPD patterns.

Primer CCTTGACTCA gave no amplification in both of the RA patients and the healthy subject group which make it unsuffient to detect the genetic diversity in RA patients figure (6).

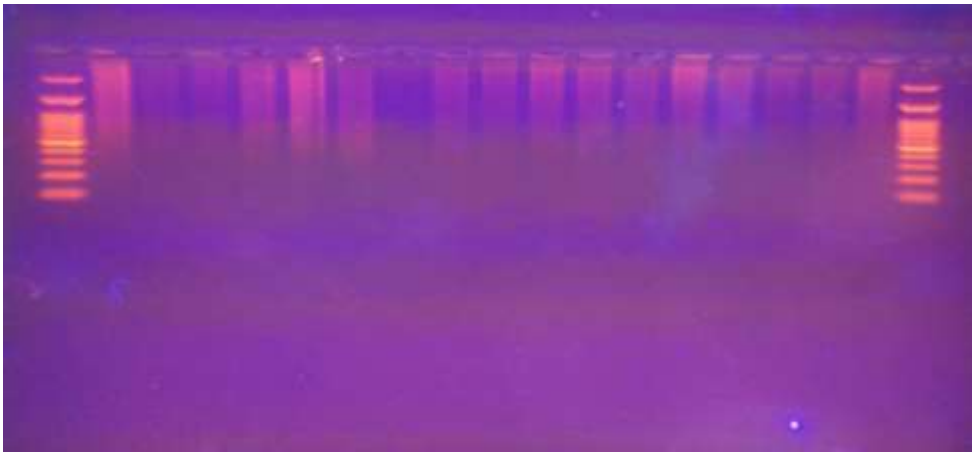

Figure 6: Gel Electrophoresis Results of Primer CCTTGACTCA on 1.5% Agarose at 50 Voltages for 3 Hours.

Primer CAGCACCCAC gave 6 different patterns as shown in figure (7).

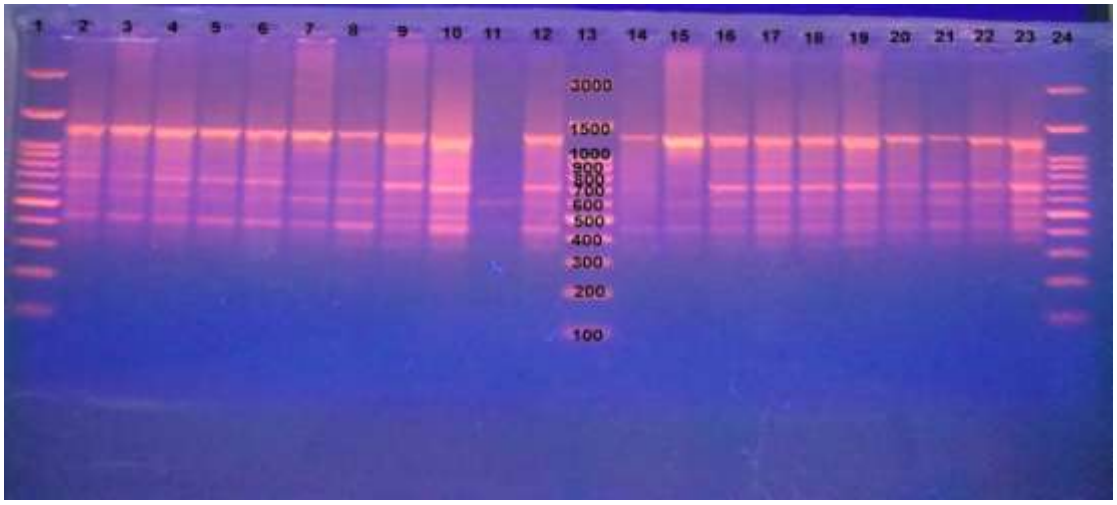

Figure 7: RAPD Pattern for Primer CAGCACCCAC on 1.5% Agarose Gel at 50 Voltages for 3 Hours

Lane 1,13 and 24 H3 100 bp DNA Ladder; Lane 2-8 RAPD patterns of healthy control subjects (H group); Lane 9-16 except13 RAPD patterns of patients using MTX as treatment (M group); Lane 17-23 RAPD patterns of patients using CS as treatment (C group)

The phylogenetic tree of this primer Figure (8) and from the resulted tree we noticed the following:

The healthy subjects group had shown quite different RAPD pattern (the first cluster) from the RA patients. This pattern is repeated in all the healthy subjects without any difference and can be used in future to detect the subjects who do not have the molecular susbtiblity to develop RA.

The patients group showing good response to corticosteroids (C) group fell into 2 main clusters or branches, one of them was the most frequent pattern in these group of patients and the other repeated only in 4 patients reserving corticosteroids.

The patients group showing good response to MTX (M) group fell into 5 major clusters; the first cluster contains the largest number of patients, the second cluster contains fewer number of patients (repeated only 5 times in the studied group of patients), while the third cluster was found in only 3 patients receiving MTX, one cluster (M5) was similar in pattern to patient pattern receiving corticosteroids and, one patient with a unique pattern (single cluster) that is quite different from all the studied group.

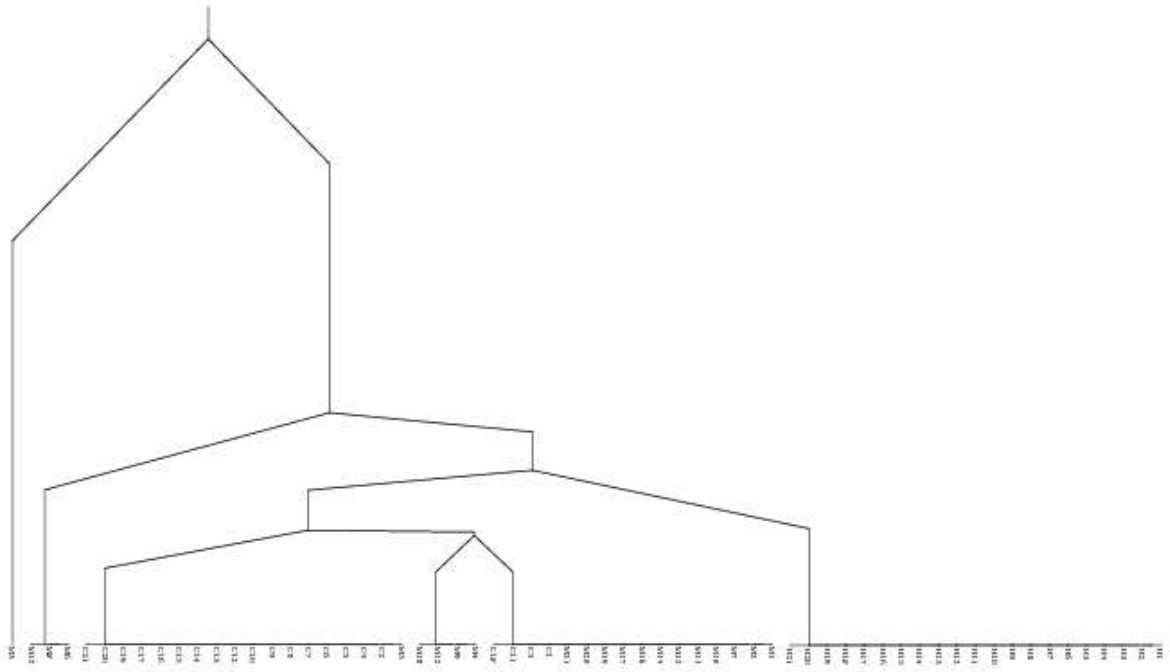

Figure 8: Phylogenetic Tree of Primer CAGCACCCAC Clustering Drawn the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) Algorithm Program.

The main reason for using of RAPD analysis is the fact that RAPD Random amplified polymorphic DNA-polymerase chain reaction is a powerful molecular marker capable of detecting DNA polymorphism based on the amplification of random DNA segments with single primer of arbitrary nucleotide sequence, and the ability of this technology to gain a large number of genetic markers that require small amounts of DNA without the requirement for cloning,

sequencing or any other form of the molecular characterization in addition to its simplicity and relatively inexpensive technique [12,13,14].

Interestingly , in the present study, we find that RAPD analysis is a very useful genetic marker that can be used in both studying the genetic diversity among Iraqi RA patients, and the association between the individual differences and the individuals´ responses to drugs used to treat RA.

Up to our knowledge either there is rare or no previous study in Iraq or in the world used RAPD technology in detection the association between the individual genome and the patients response to different treatment used to treat RA.

The only available study used RAPD in arthritis patients is the study of Mujapara and Jarullah which studied the genetic diversity among 36 Indian patients suffering from different types of arthritis which found that there was an association between random amplified DNA polymorphism pattern of the patients and the type of arthritis they have by noticing that the RAPD pattern of the rheumatoid arthritis patients are different from the pattern of osteoarthritis[15].

The primers GATGACCGCC and CAGCACCCAC showed high efficiency to detect the genetic differences among RA pateints were previously used by Zahid *et al.* in studying the genetic diversity among a group DM type 2 Iraqi patients [16].

The primer CAGCACCCAC was also used by Abed *et al.*, Ahmed *et al.*, and Ghali *et al*. to detect the genetic instability in oral squamous cell Carcinoma, breast cancer and genomic instability in blood and prostate tissues in a sample of Iraqi cancer patients resulted from cancirogenesis respectively and in all the cases showed good results in detecting the genetic differences between the tumors and the normal tissue of the same patients [17,18,19].

The primer CAGCACCCAC was also used as genetic marker to detect polymorphism that can be used for early diagnosis of breast cancer [20].

Both primers, CTGGGCAACT and CCTTGACTCA were not good in studying the genetic diversity among RA and healthy Iraqi subjects because the first primer was not able to do amplification due to the lack of its receptors, and the second primer showed similar amplification pattern in all the studied subjects which made them un probable to study the genetic diversity among the studied subjects but this finding can not be generalized on all the Iraqi population because of the limited number of subjects in this sample comparing the population study and the possible role of the geographic effect.

Analysis of the resulted pattern of amplifications revealed an important thing which is RAPD technology is an important relatively cheap and available technology can be used in pharmacogenomics for individualization or personalization the treatment for each patient by

predicting the way in which the patient will respond to different treatment before even giving it to the patients which will help both the physicians and patient in choosing the best drug for the patient in minimum time without the need to try different treatment and improving the patient quality of life and reduce the side effects.

Pharmacogenetics is a rapidly advancing area of research and holds promise that in the near future, therapy can be tailored to a given patient's genetic profile [21]. Identification of genetic determinants of drug efficacy and toxicity will be valuable because they can be ascertained in the individual patient before initiation of therapy [22].

CONCLUSION

RAPD patterns of the subjects can be used in individualization or personalization the treatment for each patient by predicting the way in which the patient will respond to different treatment before even giving it to the patients which will help both the physicians and patient in choosing the best drug for the patient in minimum time without the need to try different treatment and improving the patient quality of life and reduce the side effects.

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