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Evaluation of DNA extraction in Iraqi Rheumatoid Arthritis Patients

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ABSTRACT

The present study aims to highlight on role of DNA extraction and purity of DNA In Iraqi patients with Rheumatoid Arthritis (RA). In order to achieve that, 50 patients of both gender with RA. Another 50 individuals were selected as the Healthy control of both gender, with the age average of (20-40)years . The measured concentration and purity of DNA after DNA extraction . The obtained results after comparing patients with the healthy groups indicated DNA purity in patients and healthy control, there was no effect in RA disease in the present study. Extraction of high quality DNA with minimum time and cost will is use of interest in molecular genetic studies in Rheumatoid Arthritis patients (RA).Protein tyrosine phosphatase non-receptor type 22 (lymphoid), also recognized as PTPN22, is a protein encoded by the PTPN22 gene .

Keywords: Rheumatoid Arthritis , Purity , DNA extraction, Iraqi Rheumatoid.

INTRODUCTION

Rheumatoid arthritis is an autoimmune inflammatory disorder [**1**], that during a short time can quickly develop into severe and disabling **[2**]. The synovial joints are the main joints that influence by this disease and suffer from pain, irregularity and loss of function [**3**]. The worldwide prevalence of rheumatoid arthritis is estimated to differ between 0.5 to 1.5% which affected by geographic locations [**4]**. There is no explanation of the reason for the geographic variances [**5**]. Early diagnosis is a solution for controlling rheumatoid arthritis, in order to avoid joints damage, extra-articular complications, to avoid expensive medical therapy and surgical processes, and to prevent the functional limitation to be developed [**6**]. In spite of the fact that rheumatoid arthritis is considered as an autoimmune disease, information of its pathogenesis is unclear. It is possibly a multi-factorial disease which arises as a result of many risk factors. There is considerable evidence about involvement of important genetic component and a substantial portion of this risk appears to lie in the presence of class II allele human leukocyte antigen (HLA-DRw4) [**7**], variants of PTPN22 and other genes have also been recognized as risk factors for rheumatoid arthritis [**8**]. This insight could be of interest to diagnosing physicians and rheumatologists as swollen joints and the presence of ACPA may not be the most useful factors in identifying the severity of disease [**9**]. Further research should be enacted to determine the varying severity levels of seropositive versus seronegative RA and their criteria for diagnosis. The second component that adds to the complexity of the development and progression of RA is the interaction between the body's immune response to environmental factors. Smoking, infectious agents, periodontal disease, the gastrointestinal microbiome, and adverse life events are all related to the onset of rheumatoid arthritis [**10**]. Reports indicate that smoking, one of the strongest known risk factors for RA, may perpetuate the production of citrullinated proteins, resulting in the subsequent development of ACPAs . The interaction between what the body experiences externally and how it reacts internally increases the variance in predictors for disease activity and complicates the 5 understanding of the pathogenesis of RA . As new research and information regarding the biological and environmental factors that impact the disease become available, a better understanding and appreciation for the chronic, and sometimes invisible, effects of RA are likely to result [**11**].Extraction of high quality DNA with minimum time and cost will is use of interest in molecular genetic studies in Rheumatoid Arthritis patients (RA) [**12**]. Protein tyrosine phosphatase non-receptor type 22 (lymphoid), also recognized as PTPN22, is a protein encoded by the PTPN22 gene [**13**].

Methods

Patients

The blood samples were collected from 100 people both gender (male and femal) who attended Al Bagdad medical city, from September, 2020 to January, 2021. The samples were divided into 50 from patients average age (25-65) years and 50 from healthy control (20-40) years.

Sample collection

Take peripheral blood used to be withdrawn from each man or woman .Two milliliters of fresh blood were poured into a tube containing EDTA for DNA extraction and molecular study.

Methods

DNA extraction

Quick-gDNA™ Blood MiniPrep Catalog Nos. D3072 & D3073

Protocol

The following method for the purification of DNA from 100 µl whole blood (the volumes can be adjusted up to 200 µl (max.) depending on the requirements). Fresh, frozen, or preserved blood (in EDTA, citrate, or heparin) can be used. If material cannot be processed immediately, the sample can be "stabilized" for later processing (as noted below) although the immediate processing of blood samples is recommended.

1. A volume (100 µl) of blood with 400 µl of Genomic Lysis Buffer (1:4). Mixed completely by using vortexing 4-6 seconds, then leted stand 5-10 minutes at room temperature.

2. The mixture was transfered to a Zymo-Spin IIC™ Column2 in a collection Tube.Then, centrifuge at 10,000 x g for one minute. Discarded the collection Tube with the go with the flow through.

3. Added (200 µl) of DNA Pre-Wash Buffer to the spin column. After the transfered of the Zymo-Spin™ IIC column to a new collection tube,centrifuge at 10,000 x g for one minute.

4.In the spin column, added 500 µl of g-DNA wash buffer, then centrifuge at 10,000 x g for one minute

5.The spin column was transfered to a clean microcentrifuge tube. Added ≥50 µl DNA Elution Buffer or water to the spin column3. Incubated 2-5 minutes at room temperature after up centrifuge at top speed for 30 seconds to elute the DNA. The eluted DNA can be used immediately for molecular based applications or stored ≤-20ºC for future use.

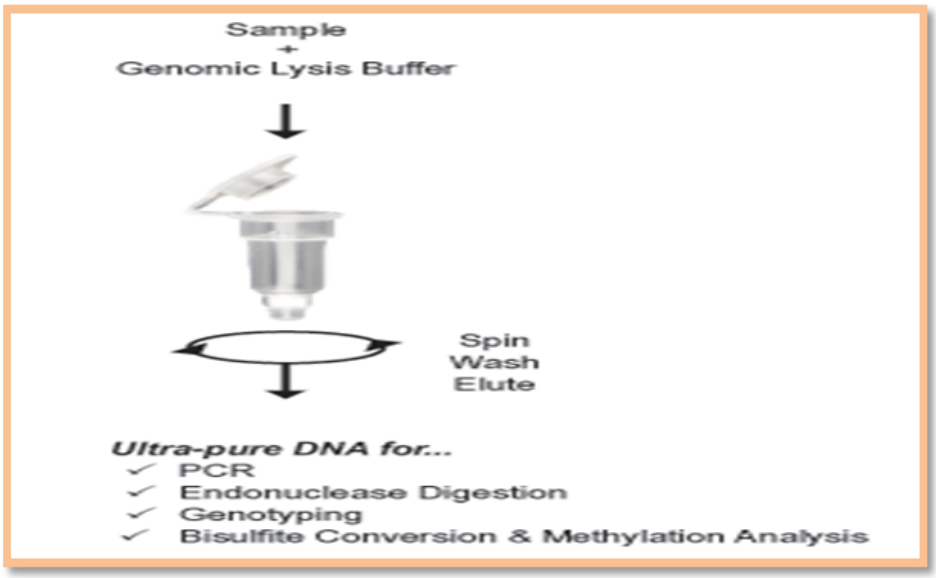

Figure 1: Steps of DNA extraction

Preparation of the Agarose Gel

According to Sambrook *et al* (1989) [**14**,**15**], the agarose gel has been made in 1.5% condensation by melting 1.5 g of agarose in 100 ml of previously made TBE buffer. Agarose has been heated to boil then left to cool down at (45-50°C).

The gel has been poured in the pour plate in which the plate of agars support was organized after fixing the comb to make holes that would preserve the samples. The gel was poured gently no longer to make air bubbles and left 30 minutes to cool down. The comb was eliminated gently of the stable agarose. The plate was constant

to its stand in the Electrophoresis horizontal unit represented by means of the tank used in the Electrophoresis. The tank was filled with TBE buffer in which it covered the gel surface.

Preparation of Sample

A 5µl of the supposed DNA was mixed to be electrophoresis (loading dye) with 3 µl of the processor loading buffer (Intron / Korea), after the mixing process, the process of loading was now to the holes of the gel. An Electric current of 7 v\c2 was exposed for 1-2 h till the tincture reached to the other side of the gel. The gel was tested by a source of the UV with 336 nm after put the gel in pool contain on 30µl Red safe Nucleic acid staining solution and 500 ml from dist. Figure (2) show the working of electrophoresis system. [**16**]

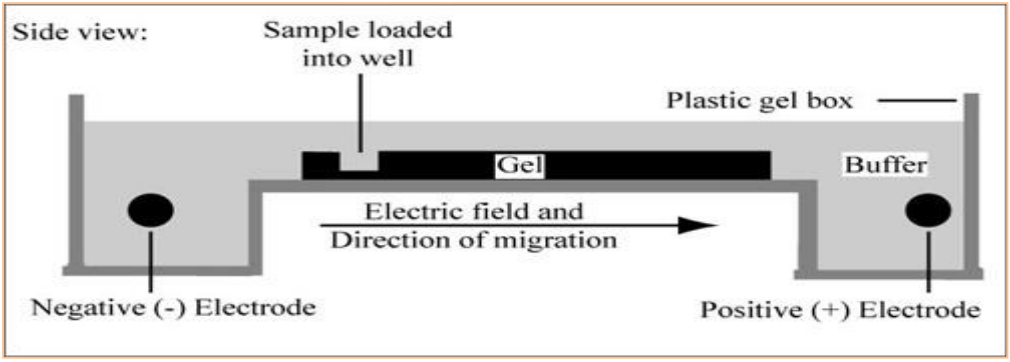

Figure 2: Working of Electrophoresis System

Determination of DNA Concentration:

Procedure:

Add 200 µl from Tris-EDTA (T.E.) to 3,800 from D. water the mix 4000 µl, pull 10 µl ignore it and add 10 µl from the dye (DNA Dye)

Pull 200 µl of the mix for each sample.

The series of the following tubes are prepared as follows:

Make a vortex mixing to a second.

Leaves on rake at room temperature for 5 min.

Extracted the value from the device immediately

Absorb260nm / Absorb280nm

Reagent	Blank	Standard	Sample
Mix	200 µl	200 µl	200 µl
DNA Extraction		2 µl	2 µl

Determination Purity of DNA

The purity of DNA was determined according to the following:

Statistical Analysis

Using the software SPSS program version 20 and excel application to find the result.

RESULTS

Molecular study

The genomic DNA extraction was done using the commercial Kit (ZYMO research™ gDNA kit, USA) from the whole blood cells. All samples (patients and controls) showed clear and sharp bands. The nucleic acid concentration and purity ratio were automatically calculated by nanodrop software, and the results were as follows (1.8- 2 ng/µl). As shown in figure 3.

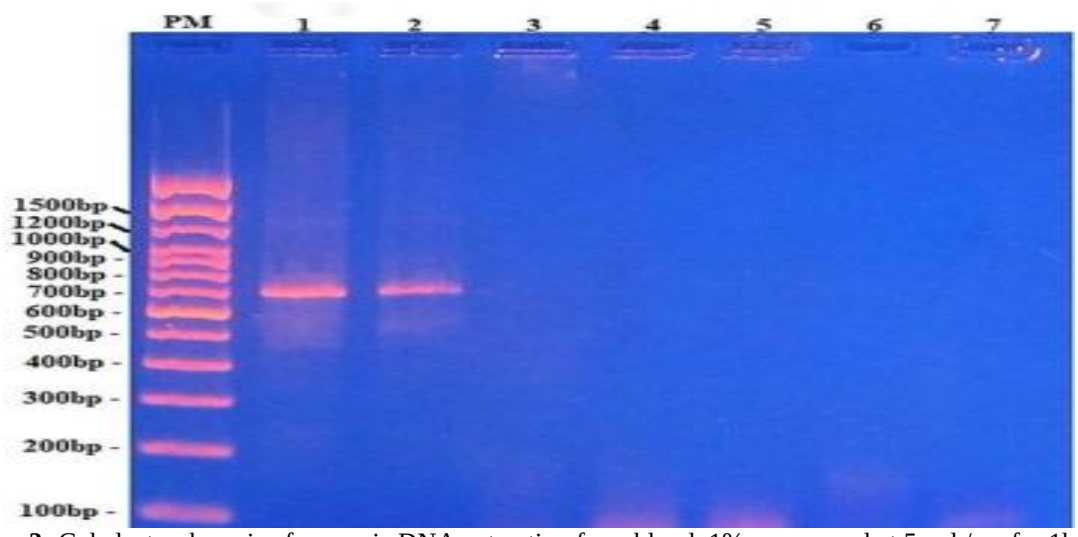

Figure 3: Gel electrophoresis of genomic DNA extraction from blood, 1% agarose gel at 5 vol /cm for 1houre.

Determination of purity DNA

The results of purity of DNA in Table 1 showed no significant ($p>0.05$) Of the patient compared with all control groups proved this study of purity of DNA not effected in the RA. Mean ±SD for patients and control (1.83±0.01 , 1.84±0.004) respectively.

Table 1: Mean and S.D. for purity of DNA for patients and control

Groups	Mean	SD
Patient	1.83	0.01
Control	1.84	0.004

DISCUSSION

Polymerase Chain Reaction Test (PCR Test) is a test in which small pieces of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) are amplified to help analyze short sequences of DNA or RNA even in samples that contain a small amount of DNA. Countless copies of DNA can be produced, and it takes only a few hours.this result was agreement with [**17**] who found PCR analysis is used to diagnose genetic diseases, or rapid diagnosis of infectious diseases, detect bacteria and viruses, identify pathogens, and perform DNA fingerprinting. PCR analysis is also used in forensic medicine to identify criminals, or early diagnosis of some types of cancer. Result was agreement with [**18,19,20**] The PCR test technique relies on amplifying a portion of DNA in order to detect it. The sample is first heated in order to separate the DNA into two strands of DNA. A specific enzyme called Taq Polymerase builds each strand to be new DNA, meaning that the DNA is copied so that each molecule contains an old part of DNA and a new part. It is worth noting that a thermal cycler is used, which is programmed to change the temperature to suit the analysis steps. It takes a few hours to perform a PCR analysis and for the results to appear. Denaturation: Denaturation occurs at 94°C, where the double-stranded DNA opens into two pieces of single-stranded DNA. Annealing: Annealing occurs at a medium temperature of 54°C, where the polymerase enzyme begins to copy. Extension: Extension occurs at 72°C, where the new DNA strand is paired with the original. There are no risks when performing a PCR analysis, as the PCR technique is sensitive, but it is worth noting that any contamination of the sample, even if it is simple, may lead to incorrect results, and sometimes it may lead to mutations in the generated part. The interpretation of PCR test results depends on the pathogen that the healthcare provider is trying to detect through the PCR test. PCR can only be used to determine the presence or absence of a known pathogen or gene. A positive result indicates the pathogen, and a negative result indicates the absence of the pathogen.

CONCLUSION

It was found that when measuring DNA purity in patients and healthy control there is no effect in R.A. disease in present study. The findings revealed a strong association between PTPN22 and RA susceptibility, in addition to a correlation between PTPN22 gene and disease activity and severity

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