International Journal of Medical Toxicology & Legal Medicine **Volume 27, No. 4S, 2024**
e-ISSN: 0974-4614
p-ISSN: 0972-0448
https://doi.org/10.47059/ijmtlm/V27I4S/021

Detection of PCR using DNA Iraqi Patients with Osteoporosis

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Received: 17.09.2024 Revised: 14.10.2024 Accepted: 04.11.2024

ABSTRACT
Osteoporosis causes bones to become weak and brittle, so much so that even a fall or minor stress, such as bending over or coughing, can cause a fracture. Fractures associated with osteoporosis are most common in the hip, wrist or spine. Osteoporosis affects men and women of all races. But white and Asian women, especially those who are postmenopausal, are at greatest risk. Medications, a healthy diet, and weight-bearing exercise can help prevent bone loss or strengthen already weak bones. Sex hormones decreased sex hormones are likely to lead to weak bones. The drop in estrogen levels in women after menopause is one of the strongest risk factors for osteoporosis. Prostate cancer treatments that reduce testosterone levels in men and breast cancer treatments that reduce estrogen levels in women also likely accelerate bone loss. The present study aims to highlight on role of DNA extraction and purity of DNA in Iraqi patients with osteoporosis. In order to achieve that, 50 patients of female with osteoporosis the age average (25-65) years. Another 40 individuals were selected as the healthy control of female, 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 osteoporosis disease in the present study.

Keywords: Osteoporosis, DNA extraction, Iraqi patients**.**

INTRODUCTION

Osteoporosis is a disease accompanied by decreasing bone mass, defects in the microarchitecture of the bone tissue and a raised risk of fragility fractures [**1**]. The causes of osteoporosis are genetic, environmental factors, and the interactions between them. Clinically osteoporosis is determined via the measurement of BMD [**2**]. Bone densitometry allows quite accurate and precise measurement of bone mass, particularly its mineral density [bone mineral density (BMD) in g/cm^2 of projected bone area. BMD accounts for 60 to 80% of bone mechanical resistance. [**3, 4**]. According to the World Health Organization (WHO), a densitometric diagnosis of osteoporosis should be based on BMD measured by dual-energy x-ray absorptiometry (DXA), compared to the mean BMD in young normal adults of the same sex (peak bone mass). The unit of measurement is the standard deviation (SD) above or below the mean peak bone mass (T-score) [**5, 6**] . It has been reported that fracture risk begins to increase exponentially at a T score <-2.5 SD, which has been established by the WHO as the cut-off for diagnosing osteoporosis. Bone densitometry is therefore the diagnostic test for osteoporosis and fracture risk assessment, just as blood pressure measurement is used to diagnose arterial hypertension and assess the risk of stroke [**7, 8**]. Anti-osteoporotic drugs target either reduced bone remodeling or stimulate bone construction in order to increase bone strength and prevent fractures [**9**]. It is important to know their potential interactions on the fracture healing process and to assess their ability to promote bone healing, most preclinical studies, largely involving osteoporotic rodent models, have demonstrated a stimulation of fracture healing by bone-forming agents; there is no evidence of any deleterious effect on the early stage of fracture healing by anti-resorptives drugs. [**10,11**]. Inhuman, several case reports and well-designed clinical trials seem to confirm the potential beneficial effects of bone-forming agents on fracture repair, more studies are needed to evaluate this systemic approach of enhancing fracture repair, especially in people diagnosed with osteoporosis [**12**]. Selection of an appropriate method for the rapid, safe and cost effective isolation of pure genomic DNA (gDNA) is a prerequisite for large-scale genetic epidemiological studies; substantial quantities of high molecular weight DNA are required for repetitive genotyping analysis, capable of withstanding prolonged storage [**13**]. The use of filtration isolation of nucleic acids (FINA) as a viable method for point-of-care extraction of leukocyte DNA from whole blood for detection of the osteoporosis by PCR with high degrees of sensitivity and specificity has been demonstrated previously . All the methods of DNA extraction consist of three basic steps: first, lysis of the

cell membrane; second, separation of the DNA from other cellular components especially protein and RNA; finally, DNA precipitation.[**14**]. Therefore, in order to fulfill the demand of a rapid and cost effective procedure for obtaining high quality genomic DNA, hereby we have aimed to develop a protocol free from costly enzymes and toxic organic solvents for extracting pure DNA from fresh and frozen human blood sample.

MATERIAL AND METHODS

The blood samples were collected from 90 people both gender (female) 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 40 from healthy control (20-40) years.

Sample blood collection

Five ml of blood were collected from both groups with anticoagulant (EDTA) tube for molecular study store at –20°C until use.

Methods

DNA extraction

Genomic DNA was extracted using Quick-gDNA™ Blood MiniPrep (Zymo/USA) rol groups were extracted by using DNA extraction kit according to the leaflet of kit; (Special protocol frozen Blood) [**15**].

Four hundred µl of Genomic Lysis Buffer was added to 100 µl of blood. Mix completely by vortexing 4-6 seconds, then leted stand 5-10 minutes at room temperature.The mixture was transferred to a Zymo-Spin IIC™ Column2 in a Collection Tube. Centrifuge at 10,000 x g for one minute. Discard the Collection Tube with the flow through. The Zymo-Spin™ IIC Column was Transferred to a new Collection Tube. and 200 µl of DNA Pre-Wash Buffer add to the spin column. Centrifuge at 10,000 x g for one minute. Five hundred µl of g-DNA Wash Buffer was added to the spin column. Centrifuge at 10,000 x g for one minute.The spin column was Transferred to a clean micro-centrifuge tube. Then Add ≥50 µl DNA Elution Buffer or water to the spin column. Incubate 2-5 minutes at room temperature and then 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

Preparation of Agarose

The material that used in preparation of gel electrophoresis is: 1X TAE buffer, loading dye, Ethidium bromide (10 mg/ml).

1- One hundred ml of 1X TAE was taken in a beaker.
2- One gm (for 1%) agarose is added to the buffer.
3- The solution was heated to boiling (using Micro Wave) until all the gel particles were dissolved.
4- One µl of Ethidium bromide (10mg/ml) was added to the agarose. The agarose was stirred in order to get mixed and to avoid bubbles. The solution was allowed to cool down at 50-60 ºC.

Casting of the Horizontal Agarose Gel

The agarose solution was poured into the gel tray after both the edges were sealed with cellophane tapes and the agarose was allowed to solidify at room temperature for 30 minutes. Carefully removed and the gel was placed in the gel tray. The tray was filled with 1X TAE-electrophoresis buffer until the buffer reached 3-5 mm over the gel [**16**].

DNA loading

Two microliter of loading dye applied to each five 5 µl DNA sample, and samples were added carefully to the individual wells. Electrical power was turned on at 100v /mAmp for 75min. DNA moves from Cathode to plus Anode poles. The Ethidium bromide stained bands in gel were visualized using Gel [**17**]. (**Figure 1, 2**).

Figure 1: shows DNA loading for Iraqi patients with osteoporosis samples

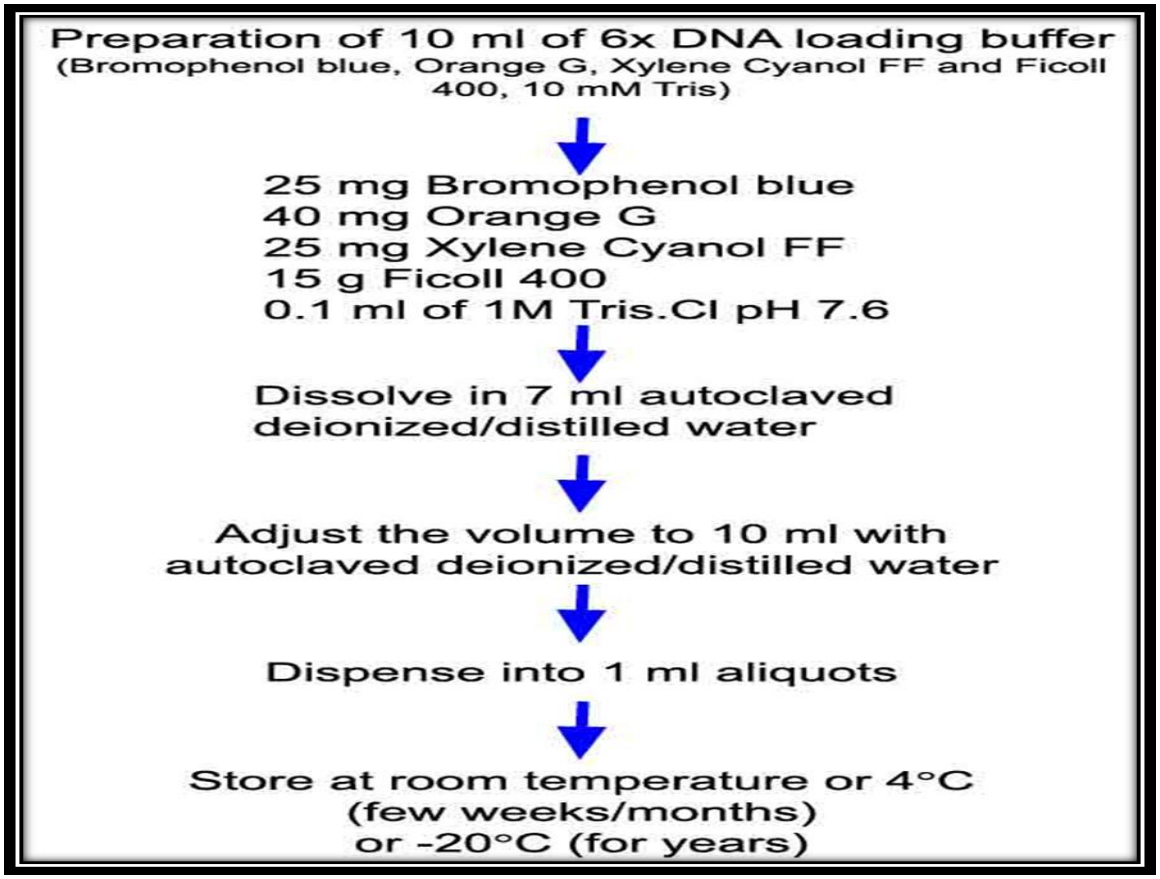

Figure 2: shows DNA loading preparation with osteoporosis samples

Statistical Analysis

The significance of differences among groups in survival, DNA coring, and electrophoresis picture was analyzed and graphically presented using Prism(Prism6.0, Graph Pad Software Inc.; Sandi ego, CA) with a significance level ofP,0.05. [**18**]

RESULTS AND DISCUSSION

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 as shown in (**Figure 3**).

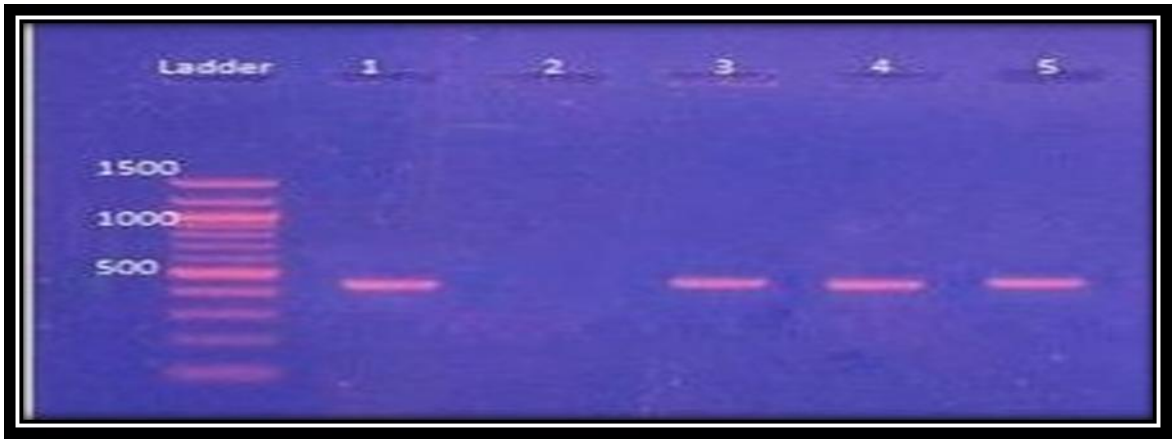

Figure 3: Gel electrophoresis of DNA extraction from blood, 1% agarose gel at 5 vol /cm for 1hour

This study was agreement with [**19**], how found, no matter how much one takes care of their body, in some cases; they may face health problems that negatively affect bone health in the early period. One of these health

problems is osteoporosis, better known as osteopenia. Osteoporosis is defined as a condition in which bones become more fragile and sensitive due to a decrease in bone mass. Bone health is one of the most important organs that maintain bone health, and it is very important in aging, and it is the most important organ that keeps the body standing, and it is necessary to have regular bone examinations to maintain bone health. At the same time, early diagnosis is very important for early treatment of osteoporosis (brittle bones).

This study was disagreement with [**20**], Osteoporosis is a disease that generally develops silently and goes unnoticed. For this reason, osteoporosis does not come to mind in cases of fractures. Because patients usually find it difficult to recognize the seriousness of this condition unless the most obvious symptoms are noticed. However, the basis of fractures in individuals over 50 years of age is mostly due to osteoporosis. With the onset of osteoporosis, bones become more susceptible to fracture. Short stature: One of the common symptoms of osteoporosis is short stature. This shortness is usually more than 3 centimeters. Humpback: The hump that appears in the advanced stages of osteoporosis significantly affects the quality of life negatively. This is because the humpback leads to compression in the abdominal area. As a result of this compression, the abdomen moves forward, the intestines are compressed and pressure occurs in the thigh due to swelling. This change in the body causes pain. Increased body pain: One of the symptoms of bone resorption is severe pain. This pain occurs mostly in the back and waist area. In case of pain that restricts movement, it is useful to consult a health institution. Because in the case of pain that restricts movement, there is a high possibility of bone fractures.

This study was agreement with [**21,22**], Osteoporosis is a treatable disease. The goal of treatment is to improve bone quality and strengthen bones. In this way, fractures can be prevented. Osteoporosis treatment usually begins with medications that reduce bone destruction and increase bone formation. In order to be able to use these medications, the individual's urine and blood tests must be evaluated and appropriate results found. In order to obtain positive results from the treatment, it is important that the treatment continues for at least one year. It is important to carry out the necessary controls at the end of one year in order to determine whether the treatment will continue or not. Osteoporosis medication is determined in line with the examinations that will be carried out by the physician specializing in the use of the medication. Because not every osteoporosis medication is suitable for every patient. In addition to medications, vitamin D and calcium supplements are also needed in the treatment of osteoporosis. Supportive therapy is very effective in achieving positive results. However, the vitamins used in these supportive therapies must be adjusted individually.

CONCLUSION

Including physical activity in daily routine is an important step in the treatment of osteoporosis. At this stage, it is useful that the activities to be preferred are aimed at strengthening the muscles. Because strengthening the muscles is effective in renewing bone mass. Brisk walking and light jogging are recommended supportive activities in the treatment of osteoporosis. Even if it is not possible to do it every day, it is recommended to do it for 20-30 minutes 3 times a week, especially outdoors. It is useful to prefer open areas rather than closed places for walking. In this way, vitamin D can also be taken naturally. In addition to jogging and walking, dancing and yoga are activities that support the treatment of osteoporosis. As with the use of medications and vitamins, the doctor's recommendation is important for physical activities. Therefore, this important decision should be made with the doctor or the activities should be done under the supervision of the doctor. It was found that when measuring DNA purity in patients and healthy control there is no effect in osteoporosis disease in present study.

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