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# COMPARISION OF SILICA GEL AND SPIN COLUMN METHOD USED FOR DNA ISOLATION OF MULTIPLE HEREDITARY EXOSTOSES AFFECTED PROBAND

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**ABSTRACT**

Multiple Hereditary Exostoses (MHE) is a very rare kind of disorder having occurrence as 1/50000 individuals. MHE is a medical condition in which multiple bony lumps that develop on the bones of patient.this condition is formed due to abnormal gene product of *EXT1* gene forming abnormal exostosin type1 protein. The *EXT1* gene loci has been identified at chromosome 8q23-24, 5.This study was an attempt to standardize the protocol required for isolation of the DNA from such affected patients, further which could be used for PCR mutation detection studies. In the present study DNA is isolated from patients sample using silica gel and spin column method. These two techniques are used commercially for DNA isolation process from whole blood cells. The comparative data of the DNA isolated from MHE probands and further confirm that DNA yield obtained using silica gel is higher than spin column method.

**Keywords: Multiple Hereditary Exostoses, Spin Column, *EXT*1 Gene, Exostosin, Silica Gel**

## INTRODUCTION

In the United States, the terms "exostoses" and "hereditary multiple exostoses" have been used to denote the growths and the disorder, but the World Health Organization (WHO) has selected the nomenclature "osteochondromas" for exostoses and "multiple osteochondromas" for the disorder **[1, 2]**. Hereditary Multiple Exostoses (HME) is a rare medical condition in which multiple bony spurs or lumps (also known as exostoses, or Osteochondromas) develop on the bones of affected patient. This disorder has an estimated prevalence of 1/50,000, making it one of the most frequent skeletal dysplasias **[3-7]**. Osteochondromas are rarely present at birth, but in more than 80% of the patients they develop gradually during the first decade of life and increase in size until closure of the growth plates at the end of puberty **[8-11]**.

HME can lead to the shortening and bowing of bones; affected individuals often have a short stature. MHE can cause pain to people of all ages. To children, this can be especially painful. During exercise, it can cause a lot of pain. Depending on their location the exostoses it can cause the following problems: pain or numbness from nerve compression, vascular compromise, inequality of limb length, irritation of tendon and muscle, as well as a limited range of motion at the joints upon which they encroach **[7, 12-20]**. MHE can lead to the shortening and bowing of bones and affected individuals often have a short stature. The proportion of individuals with multiple hereditary exostoses who have clinical findings increases from approximately 5% at birth to 96% at age 12 years. By adulthood, 75% of affected individuals have a clinically evident bony deformity. Males tend to be more severely affected than females. Most commonly involved bones are the femur (30%), radius and ulna (13%), tibia (20%), and fibula (13%). Hand deformity resulting from shortened metacarpals is common **[6]**.

The most serious complication of MHE is sarcomatous degeneration of an osteochondroma. Axial sites, such as the pelvis, scapula, ribs, and spine, are more commonly the location of degeneration of osteochondromas to chondrosarcoma **[12, 15-19]**.

About 200 mutations in the *EXT*1 gene have been identified in people with hereditary multiple exostoses type 1.Most of these mutations are known as "loss-of-function" mutations, as they prevent any functional exostosin-1 protein from being made. The loss of exostosin-1 protein function prevents

it from forming a complex with the exostosin-2 protein and adding heparan sulfate to proteins. It is unclear how this impairment leads to the signs and symptoms of hereditary multiple exostoses **[13]**. To understand a bit about the impairment, the primary step involves successful isolation of DNA from peripheral blood cells of a MHE affected proband. Availability of adequate high quality genomic DNA is essential to succeed in various molecular biological techniques such as sequencing, cDNA synthesis and cloning, RNA transcription, nucleic acid labeling random primer labeling) etc **[14]**. Hence extraction of high quality DNA is essential to carry out further analysis in molecular biology. In the present study isolated DNA from MHE proband and normal person using two different protocols were compared and analyzed.

## MATERIALS AND METHODS

Multiple Hereditary Exostoses affected patients are identified and their consent form is filled up as per the format and guidelines given by Medical Council of India. The ethical clearance for the research study is obtained from the Institutional Ethics Committee. Blood Sample of the identified patients is collected with the help of a certified lab technician in EDTA bulbs (2ml). DNA was isolated from the whole blood cells using two different methods i.e. Silica gel method and Spin Column method.

### Spin Column Method – Protocol I

This method uses a specially treated glass filter membrane fixed in to a column to efficiently bind DNA in the presence of high salts. Blood lysis buffer, wash buffer, elution buffer and proteinkase K is used for DNA isolation. Small volume of blood (200µl) is taken in a centrifuge tube and 200µl of BLB and 20µl of proteinase K is added to it. The tube is kept in water bath for 10 minutes at 65°C. 200µl of ethanol is added to it to obtain a homogenous solution. The sample is then transferred into column assembled in a collection tube and spinned at 6,500rpm for 1 minute. The flow through is discarded. The column is then washed with WB-1 and WB-2 for 1 minute and 5 minutes respectively at 6500 and 13,500rpm. Each time discarding the flow through. The column is then placed in a clean centrifuge tube and 100µl of EB is added to elute the DNA. The eluted DNA is stored at 4°C

### Silica Gel Method- Protocol II

In this method DNA molecules bind to silica surfaces in presence of high salts. Plasma is separated from blood cells in the first step and erythrocytes are lysedby a hypotonic shock of distilled water washes. Extraction buffer (guianidiumthiocyanate and Triton X) is

added further which facilitates the denaturing of proteins, including RNAses and separates rRNA from ribosomes.Triton X is a detergent which solubilizes lipids and causes cells to lyse releasing DNA into solution. A DNA molecule then binds to the silica particles assed to the solution under high salt concentration. The silica with absorbed DNA is then subjected to cleansing with wash buffer which helps to remove salts and impurities from the original sample. Ethanol and isopropanol is used to precipitate the DNA molecule which is eluted and stored in TE.

## RESULTS AND DISCUSSION

DNA isolated from MHE affected probands were subjected to Agarose gel electrophoresis.

DNA isolated was analyzed using two methods: UV-Spectrophotometric method and Using Qubit 2.0 Fluorometer.

The recorded data is given below in terms of graphical representations **(Figure 1, 2)** and tabulations **(Table 1, 2)**.

### UV- Spectrophotometer Analysis

**Table 1: UV- Spectrophotometer Analysis**

| DNA ISOLATION METHOD | | Absorbance at | | Measured DNA concentration (µg/µl) | Ratio of (260/280) |
|---|---|---|---|---|---|
| | | 260 nm | 280 nm | | |
| PROTOCOL I- Spin Column | NORMAL | 0.587 | 0.325 | 0.029 | 1.81 |
| | PATIENT | 0.33 | 0.184 | 0.0165 | 1.793 |
| PROTOCOL II – Silica Gel | NORMAL | 0.328 | 0.175 | 0.0164 | 1.874 |
| | PATIENT | 0.299 | 0.164 | 0.01495 | 1.823 |

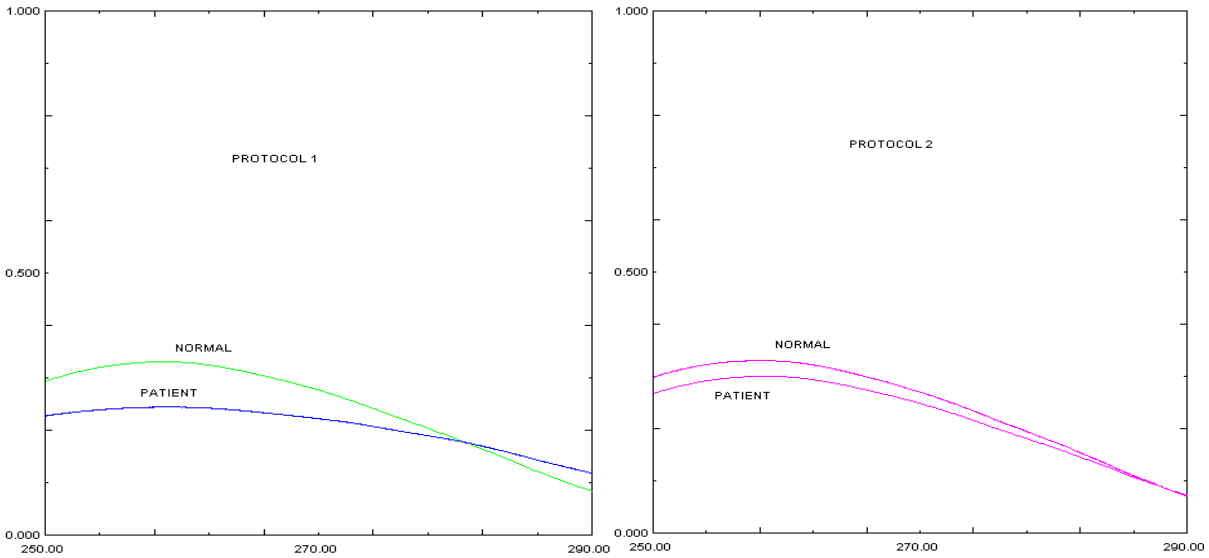


**Figure 1: Graphs of DNA Sample Isolated Using a) Protocol I, b) Protocol II**

**Qubit 2.0 Fluorometer Analysis**

The DNA was quantified on Qubitfluorometer. The following is the recorded data for the four patient's samples.

The graph in the UV- Spec analysis shows differences in the amount of DNA sample extracted during the process. The DNA isolation done using Silica gel method yields pure DNA while the DNA sample extracted using spin column method shows bit contamination of proteins which can observed by the readings obtained in the $A_{260/280}$ absorbance ratio readings.

The Graphical representation of the concentration of DNA obtained using Qubitfluorometer also states that DNA isolated using silica gel technique yields more amount of DNA than using Spin column technique.

**Table 2: DNA Quantification Using Qubitfluorometer**

| | | Concentration of DNA (*ng*/ µl) | Total Volume Present (µl) | Total Concentration of DNA (µg) |
|---|---|---|---|---|
| **Protocol II Silica Gel** | **Normal** | **160** | **200** | **32** |
| | **Patient** | **160** | **200** | **32** |
| **Protocol I Spin Column** | **Normal** | **100** | **200** | **20** |
| | **Patient** | **105** | **200** | **21** |

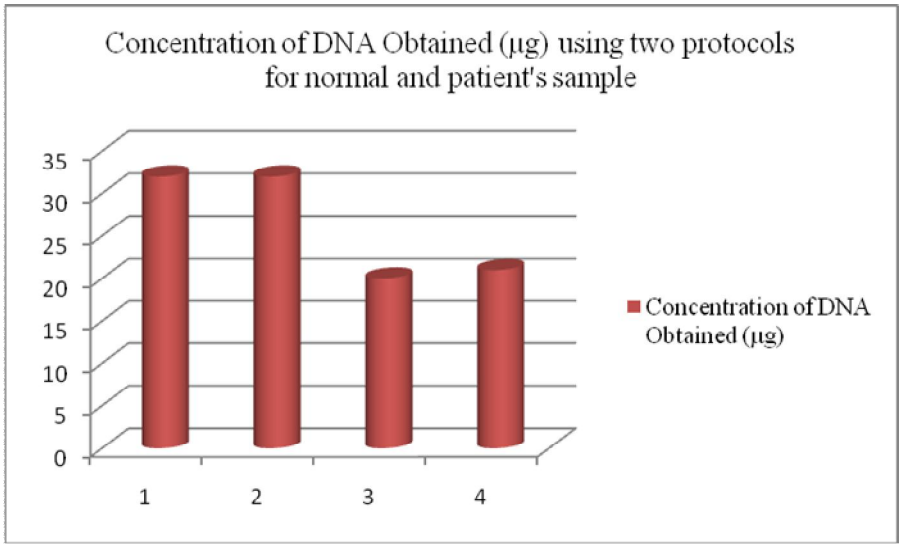


**Figure 2: DNA Quantification Using Qubit- Fluorometer**

## CONCLUSION

It is hereby concluded from the present studies that DNA isolation using silica gel technique yields more quantity of DNA as compared to the spin column technique in a Multiple Hereditary Exostoses affected proband.

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**REFERENCES**

**[1]** Kivioja A, Ervasti H, Kinnunen J, Kaitila I, Wolf M and Böhling T, Chondrosarcoma in a family with multiple hereditary exostoses, The J. Joint and Bone Surgery, 82-B (2), 2000.

**[2]** Bove´e JVMG, Cleton-Jansen AM, Kuipers-Dykshoorn NJ, Van Den Broek LJCM, Taminiau AHM, Cornelisse CJ and Hogendoorn PCW, Loss of heterozygosity and DNA ploidy point to a diverging genetic mechanism in the origin of peripheral and central chondrosarcoma, Genes Chromosomes Cancer, 26 (3), 1999, 237-46.

**[3]** Chen Niu, HirutKebede, Dick L Auld, Jason E Woodward, Gloria Burow, and Robert J Wright, A safe inexpensive method to isolate high quality plant and fungal DNA in an open laboratory environment, Af. J. Biotechnol., 7 (16), 2008, 2818-2822.

**[4]** Das S, Tiwari KL, Sen S and Singh A, Rapid One step DNA Extraction Method from *Gastrimargus Musicus*) through Formaldehyd*e*, J. Pharmaceu. and Biomedical Sci., 20 (20).

**[5]** Frostegard A, Courtois S, Ramisse V, Clerc S, Bernillon D, Le gall F, Jeannin P, Nesme X and Simonet P, Quantification of Bias Related to the Extraction of DNA Directly from Soils, Appl. and Environ. Microbiol., 0099-2240/99/$04.0010 Dec. 1999, 5409-5420.

**[6]** Glasel JA, Validity of nucleic acid purities monitored by 260 nm/280 nm absorbance ratio, Biotechniques, 18, 1995, 62-63.

**[7]** Geraldine R Vink, Stefan J White, Strelicija Gabelic, Pancras CW Hogendoorn, Martijn H and Breuningand Egbert Bakker, Mutation screening of EXT1 and EXT2 by direct sequence analysis and MLPA in patients with multiple osteochondromas: splice site mutations and exonic deletions account for more than half of the mutations, European J. Human Genetics, 13, 2005, 470-474

**[8]** Khosravinia H, N Narasimha Murthy, D. Thertha Parasad and N. Pirany, Optimizing factors influencing DNA extraction from fresh whole avian

blood, Af. J. Biotechnol., 6 (4), 2007, 481-486.

**[9]** Hasibe Cingilli Vural, Genomic DNA isolation from aromatic and medicinal plants growing in Turkey, Scientific Res. and Essay, 4 (2), 2009, 059-064.

**[10]** Hillis DM, Larsen A, Davis SK and Zimmer EA, Nucleic Acids III: Sequencing. In: DM Hillis, C Moritz (eds): *Molecular Systematics*, Sinauer Associates, Inc., Sunderland, Massachusetts, 1990, 318-380.

**[11]** Hosek J, Svastova P, Moravkova M, Pavlik I and Bartos M, Methods of mycobacterial DNA isolation from different biological material: a review, Veterinarni Medicina, 51, 2006 (5), 180-192.

**[12]** Ivy Jennes, Mark M Entius, Els Van Hul, Alessandro Parra, Luca Sangiorgi and Wim Wuyts, Mutation Screening of *EXT1* and *EXT2* by Denaturing High-Performance Liquid Chromatography, Direct Sequencing Analysis, Fluorescence *in Situ* Hybridization, and a New Multiplex Ligation- Dependent Probe Amplification Probe Set in Patients with Multiple Osteochondromas, J. Mol. Diagnostics, 10 (1), 2008.

**[13]** Khosravinia H and Ramesha KP, Influence of EDTA and magnesium on DNA extraction from blood samples and specificity of polymerase chain reaction, Af. J. Biotechnol., 6 (3), 2007, 184-187.

**[14]** Laws GM and Adams SP, Measurement of 8-OHdG in DNA by HPLC/EDC: The importance of DNA purity, BioTechniques, 20, 1996, 36-38.

**[15]** Palumbi SR, Nucleic Acids II: The Polymerase Chain Reaction. In: DM Hillis, C Moritz abd BK Mable (eds): Molecular Systematics, 2nd Ed., Sinauer Associates, Inc., Sunderland, Massachusetts, 1996, 205-274.

**[16]** Pedrini E, De Luca A, Valente EM, Maini V, Capponcelli S, Mordenti M, Mingarelli R, Sangiorgi L and Dallapiccola B, Novel EXT1 and EXT2 Mutations Identified by DHPLC in Italian Patients with Multiple Osteochondromas, Human Genome Variation Society, 23 (3), 2005, 280.

**[17]** Porter DE, Lonie L, Fraser M, Dobson-Stone C, Porter JR, Monaco AP and Simpson AH, Severity of disease and risk of malignant changes in hereditary multiple exostoses,

Agenotype-phenotype study, J. Bone Joint Surg. Br., 86, 2004, 1041-1046

**[18]** Johny S, Kanginakudru S, Muralirangan MC and Nagaraju J, Morphological and molecular characterization of a new microsporidian (Protozoa: Microsporidia) isolated from Spodopteralitura (Fabricius) (Lepidoptera: Noctuidae), Parasitology, 2005.

**[19]** Wang TY, Wang L, Zhang JH and Dong WH, A simplified universal genomic DNA extraction protocol suitable for PCR, Genet. Mol. Res., 10 (1), 2011, 519-525.

**[20]** Wolfram H, Ulrike H, Sibylle S, Bianca M, Christiane Z, Steffen L, Axel Bohring, Beate Mitulla, Usha Peters and Ursula G Froster, New Mutations of *EXT1* and *EXT2* Genes in German Patients with Multiple Osteochondromas, Annals of Human Genetics, 2009, 283-291