

**BIOMECHANICAL INVESTIGATION OF THE
EFFECT OF EXTRA CORPOREAL
IRRADIATION ON RESECTED HUMAN BONE**

SAKSHI CHAUHAN



**DEPARTMENT OF APPLIED MECHANICS
INDIAN INSTITUTE OF TECHNOLOGY DELHI**

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by

Sakshi Chauhan

DEPARTMENT OF APPLIED MECHANICS

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CERTIFICATE

This is to certify that the thesis entitled “**BIOMECHANICAL INVESTIGATION OF THE EFFECT OF EXTRA CORPOREAL IRRADIATION ON RESECTED HUMAN BONE**” being submitted by **Ms. SAKSHI CHAUHAN** to the Indian Institute of Technology Delhi for the award of the degree of **DOCTOR OF PHILOSOPHY** is a record of bonafide research work carried out by her under our supervision and guidance. The matter presented in this thesis has not been submitted, in part or in full to any other University or Institute for the award of any degree or diploma.

Prof. Suhail Ahmad

Professor

Department of Applied Mechanics

Indian Institute of Technology Delhi, India

Prof. Anamika Prasad

Assistant Professor

Department of Mechanical Engineering

University of South Dakota, U.S.A

Prof. Shah Alam Khan

Professor

Department of Orthopaedics

AIIMS, New Delhi, India

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Abstract

Malignant bone tumors (MBT) such as Osteosarcoma and Ewing sarcoma accounts for 3% to 8% of all cancers in paediatrics and young adults. Up until the 1970s, amputation was the only available treatment for MBT. While currently, care includes a combination of treatment including neo-adjuvant therapy, chemotherapy and surgical excision via (a) bone allograft, (b) prosthesis, and (c) ECRT. Each of the methods is important from clinical viability but has its own disadvantages such as limitation of donor and/or bone bank, cost, possibility of multiple surgeries and affordable high-quality prosthesis. In such a scenario, to use Extra Corporeal irradiation and Reimplantation Therapy (ECRT) as a biological reconstruction technique of limb salvage surgery for MBT patients offers cutting edge advantages. The “implant” being patient’s own bone is by default “patient-specific” with low risk of immunological reaction. ECRT, which was introduced in 1968 by Spira and Lubin is now an established technique actively employed in Europe and Asia. ECRT is the only available treatment in many cases such as under low-resource, and absence of the bone bank. ECRT included typical radiation dosage varying from 50Gy to 300 Gy to control or kill malignant cells. Such intentional radiations are found to be responsible for alterations in bone properties. For complete evolution of ECRT, various parameters affecting structure-function relationship needs to be considered.

Ample amount of clinical studies have been done on ECRT. However, to the best of our knowledge limited investigation exists to understand bio-mechanical response of bone post single high-dose radiation and that too on human bone. The present study is one of its kinds since it involves investigation directly on human samples. Here, 15 patients undergoing ECRT therapy at the Department of Orthopaedic, All Indian Institute of Medical Sciences (AIIMS) Delhi, India were enrolled for the study. Here, changes in strength, composition and microstructure in the bone of patients subjected to ECRT are investigated. The micro-scale

response of human bone pre-irradiation was compared with its corresponding post-irradiation response. In particular, mechanical properties of bone were investigated using instrumented indentation while surface deformation and time-dependent response were determined using Atomic Force Microscopy (AFM). The observed micro-scale mechanical response was compared and analyzed in the light of compositional behaviour obtained via Raman spectroscopy. The changes in the bone microstructure particularly bone mineral density and porosity after post-irradiation were quantified using micro-computer tomography (μ -CT).

Current work leads to the conclusion that due to irradiation there is an overall reduction in indentation modulus and hardness. Subsequently, increase in deformation, viscous response, and energy dissipation across all samples were found. The changes in mechanical properties were found to be consistent with compositional changes, where mineralization and amount of calcium content was found to be decreased. It was observed that radiation induces upto 50% decrease in amount of mineralization. Demineralization due to radiation is a major phenomenon governing the elastic stiffness and plastic deformation under single dose of 50 Gy radiation. In addition, the microstructural study via micro-CT also parallel's the compositional and mechanical findings. It establishes that bone mineral density is reduced while tissue becomes more porous post-irradiation. The study not only provides insight into bone alteration mechanism post ECRT, but also provides a quantitative value to deterioration of patient-specific bone, which can be of higher importance to clinicians in post-operative care management and ultimately to improved patient-specific outcome.

सार

घातक अस्थि ट्यूमर (एमबीटी) जैसे ओस्टियोसारकोमा और इविंग सारकोमा, पीडियाट्रिक्स और युवा वयस्कों में सभी कैंसर के 3% से 8% तक होते हैं। 1970 के दशक तक, एमबीटी के लिए विच्छेदन एकमात्र उपलब्ध उपचार था। वर्तमान में, देखभाल में उपचार का एक संयोजन शामिल है जिसमें नव-सहायक चिकित्सा, कीमोथेरेपी और सर्जिकल छांटना के माध्यम से (ए) हड्डी एलोग्राफ्ट, (बी) प्रोस्थेसिस, और (सी) ईसीआरटी शामिल हैं। प्रत्येक विधि नैदानिक व्यवहार्यता से महत्वपूर्ण है, लेकिन इसके अपने नुकसान हैं जैसे कि दाता और / या अस्थि बैंक की सीमा, लागत, कई सर्जरी की संभावना और सस्ती-उच्च गुणवत्ता वाले कृत्रिम अंग। ऐसे परिदृश्य में, एमबीटी के मरीजों के लिए निम्नांकित निस्तारण सर्जरी की जैविक पुनर्निर्माण तकनीक के रूप में एक्स्ट्रा कॉर्पोरल इर्रिडिएशन एंड रिइम्प्लिमेंटेशन थेरेपी (ईसीआरटी) का उपयोग करना अत्याधुनिक लाभ प्रदान करता है। इम्यूनोलॉजिकल रिएक्शन के कम जोखिम के साथ "प्रत्यारोपण" रोगी की अपनी हड्डी है, डिफ़ॉल्ट रूप से "रोगी-विशिष्ट" है। ईसीआरटी, जिसे 1968 में स्पाइरा और ल्यूबिन द्वारा पेश किया गया था, अब यूरोप और एशिया में सक्रिय रूप से कार्यरत एक स्थापित तकनीक है। ईसीआरटी कई मामलों में एकमात्र उपलब्ध उपचार है जैसे कम संसाधन, और अस्थि बैंक की अनुपस्थिति। ईसीआरटी में घातक विकिरण कोशिकाओं को नियंत्रित करने या मारने के लिए 50Gy से 300 Gy तक की विशिष्ट विकिरण खुराक शामिल थी। इस तरह के जानबूझकर विकिरण हड्डियों के गुणों में परिवर्तन के लिए जिम्मेदार पाए जाते हैं। ईसीआरटी के पूर्ण विकास के लिए, संरचना-कार्य संबंध को प्रभावित करने वाले विभिन्न मापदंडों पर विचार करने की आवश्यकता है। ईसीआरटी पर पर्याप्त मात्रा में नैदानिक अध्ययन किए गए हैं। हालांकि, हमारे ज्ञान का सबसे अच्छा सीमित जांच हड्डी पोस्ट एकल उच्च खुराक विकिरण की जैव यांत्रिक प्रतिक्रिया को समझने के लिए मौजूद है और वह भी मानव हड्डी पर। वर्तमान अध्ययन इसका एक प्रकार है क्योंकि इसमें मानव नमूनों पर सीधे जांच शामिल है। यहां, ऑर्थोपेडिक विभाग, अखिल भारतीय आयुर्विज्ञान संस्थान (एम्स) दिल्ली, भारत में ईसीआरटी थेरेपी से गुजरने वाले 15

रोगियों को अध्ययन के लिए नामांकित किया गया था। यहां, ईसीआरटी के अधीन आने वाले रोगियों की हड्डी में ताकत, संरचना और माइक्रोस्ट्रक्चर में परिवर्तन की जांच की जाती है। मानव अस्थि पूर्व विकिरण की सूक्ष्म पैमाने पर प्रतिक्रिया की तुलना इसके बाद के विकिरण प्रतिक्रिया के साथ की गई थी। विशेष रूप से, हड्डी के यांत्रिक गुणों की जांच इंस्ट्रूमेंट इंडेंटेशन का उपयोग करते हुए की गई, जबकि सतह विरूपण और समय पर निर्भर प्रतिक्रिया को परमाणु बल माइक्रोस्कोपी (एएफएम) का उपयोग करके निर्धारित किया गया था। देखे गए सूक्ष्म-पैमाने पर यांत्रिक प्रतिक्रिया की तुलना और विश्लेषण रमन स्पेक्ट्रोस्कोपी के माध्यम से प्राप्त संरचनात्मक व्यवहार के प्रकाश में किया गया था। माइक्रो-कंप्यूटर टोमोग्राफी का उपयोग करके पोस्ट-विकिरण के बाद हड्डी के माइक्रोस्ट्रक्चर में विशेष रूप से हड्डी खनिज घनत्व और छिद्र में परिवर्तन किए गए थे।

वर्तमान कार्य इस निष्कर्ष की ओर जाता है कि विकिरण के कारण इंडेंटेशन मापांक और कठोरता में समग्र कमी होती है। इसके बाद, सभी नमूनों में विरूपण, चिपचिपा प्रतिक्रिया और ऊर्जा अपव्यय में वृद्धि पाई गई। यांत्रिक गुणों में परिवर्तन को संरचनागत परिवर्तनों के अनुरूप पाया गया, जिसमें खनिज और कैल्शियम सामग्री की मात्रा में कमी पाई गई। यह देखा गया कि विकिरण खनिज की मात्रा में 50% तक की कमी लाती है। 50 Gy विकिरण की एकल खुराक के तहत लोचदार कठोरता और प्लास्टिक विरूपण को नियंत्रित करने वाली विकिरण की वजह से डिमिनराइजेशन एक प्रमुख घटना है। इसके अलावा, माइक्रो-सीटी के माध्यम से माइक्रोस्ट्रक्चरल स्टडी भी समानांतर और यांत्रिक निष्कर्षों के समानांतर है। यह स्थापित करता है कि अस्थि खनिज घनत्व कम हो जाता है, जबकि ऊतक अधिक छिद्रपूर्ण पश्च-विकिरण हो जाता है। अध्ययन न केवल हड्डी परिवर्तन तंत्र पोस्ट ईसीआरटी में अंतर्दृष्टि प्रदान करता है, बल्कि रोगी-विशिष्ट हड्डी के बिगड़ने के लिए एक मात्रात्मक मूल्य भी प्रदान करता है, जो कि पश्चात देखभाल प्रबंधन में चिकित्सकों के लिए अधिक महत्व का हो सकता है और अंततः रोगी-विशिष्ट परिणाम बेहतर कर सकता है।

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