Contract # TUR- 8198

STUDY OF OSTEOPOROSIS THROUGH THE MEASURMENT OF BONE DENSITY, TRACE ELEMENTS, BIOMECHANICAL PROPERTIES AND IMMUNOCYTOCHEMICALS

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Starting date of current contract: November 1, 1994

Presented at IAEA RCM on
"Comperative International Studies of Osteoporosis Using Isotpoe Techniques"
December 12-15, 1994
Vienna, Austria

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Abstract

Osteoporosis is defined as an absolute decrease in the amount of bone to a level below required for mechanical support. It is an important bone disease in elderly people in many countries. Unfortunately, there is no reliable statistical data in Turkey for the incidence of osteoporosis. A decrease in bone mass is the important cause in fractures in osteoporosis. Therefore, we intend to study both bone density and other variables such as trace elements, biomechanical properties and other immunocytochemicals in bone; all combined might give an information about the cause and prevention of osteoporosis.

1. Scientific Background and Scope of the Project

1.1 Scientific Background

As same as in other European countries, there is an increasing interest in Turkey towards the etiology of osteoporosis. Mainly two medical schools in Turkey, and some private medical centers in Ankara have established a protocol to investigate bone loss by dual energy X-ray absorptiometry (DEXA). A multicentric study in Turkey indicated that the rate of osteoporosis in post menopausal woman is not significantly different than men for the same age group, and the rate of hip fractures in the elderly population is far less than that of the developed countries [1]. Although this research was based on plain X-ray studies and retrospective data of the hip surgery cases the reason for the low rate of osteoporosis is worthwhile for further evaluation.

Heparin induced experimental osteoporosis in rabbits and its treatment by calcitonin was evaluated by compression and bending experiments at the Biomechanical Laboratory of the Department of Engineering Sciences, Middle East Technical University. Furthermore, the established osteoporosis was evaluated by vibration analyses [2]. The possible porosis of calcium hydroxyapaptite implantation into the bone was evaluated by bone densitometry and biomechanical study at the same laboratory [3]. Substance P receptors on

periosteal cells were recognized in vitro that may indicate a neuroendocrinological control on bone metabolism [4].

Determination of bone minor and trace element content is of considerable medical interest, since bone serves an important storage areas not only major elements like calcium, phosphate and magnesium, but other essential elements for the body. Excess of deficiency of some of these elements have a role in the development of bone disease such as osteoporosis, therefore, research on trace elements in bone as in diet and biological material [5-7] is very important.

1.2 Scope of the Project

The objective of the study can be summarized as;

1. Bone mineral density analyses by DEXA;
   - **a.** of randomly selected individuals of the Turkish population between the ages of 15 and 50.
   - **b.** of patients above 50 years of age.
2. Measurment of trace elements in bones;
   - **a.** of patients undergoing surgery due to any other reason than osteoporosis that fall into the age spectrum mentioned in 1.a.
   - **b.** of patients older than 50 years and suffer from a hip fracture that may be related to osteoporosis. Bone density analyses of these individuals will be performed at the time of addmitance to the hospital. The patiens will consist of the same individuals of 1.b.
3. Biomechanical studies on bones of the patients of 1.a and 1.b.
4. Immunocytochemical studies on bones of the patiens of 1.a and 1.b.

2. Methods

2.1 Bone Mineral Density Analyses by DEXA

Dual energy X-ray absorptiometry (DEXA) is a recently developed method to evaluate the bone density accurately.The rate of exposure to radiation by this method is inconsequential. The bone mass can be measured with 98 % of significance and the results are evaluated as g/cm^3 when measurements are performed in two directions. The disadvantages of the DEXA method is;

1. The fat rate of the individual may affect the end result,
2. Alterations may exist due to calibration differences between different machines,
3. If the measurements are performend in only one direction, the results can be obtained as g/cm^2.

Furthermore, the osteophytes arround the spine due to degenerative changes may alter the results.

Randomly selected individuals between the ages of 15 and 50 will be evaluated by DEXA. At least 15 subjects for each sex who are known to be free of any disease will be selected. The height, body weight and menstural cycle of the females will be questioned. The rate of exercise, smoking and alcohol usage will be recorded. The Hologic QDR 2000 bone densitometer with a mobile head will be used during these experiments. Bone densities of lumbar one to lumbar four vertebrae will be obtained in the antero-posterior and lateral projection. The hip region will be evaluated only in the antero-posterior direction. People under thyroid hormone or steroid treatment will be discharged from the study. A questionnarie for the people undergoing DEXA is presented in the appendix.

2.2 Measurement of Trace Elements in Bone

The absence of some trace elements such as calcium, magnesium, selenium and zinc, and the excessiveness of aluminum, cadmium, sodium and heavy metals may increase the rate of osteoporosis.

According to the data obtained from the literature, we propose to obtain bone biopsiesfrom conformable age grouped individualsof the bone density study. These people will be selected among patients undergoing orthopaedic surgery for any other reason than osteoporosis. The bones will be obtained from the iliac crest during autogenous graft removal. A possible protocol of gaft removal is listed below:

1. The iliac bone should be tricortical.
2. The size should be approximately 3.0 x 1 5 x 1 5 cm
3. During removal a pure titanium saw will be used, and no other metal will touch the bone to prevent metal contamination. The soft tissues and blood cells will be cleaned using pure titanium instruments and de-ionised water Prior the trace element studies the bones will be treated with carbon tetra chloride and defatted by methanol.
4. The bones will be preserved in polyethylene bags at -20°C. The inside of the bags will be rinsed with de-ionized water.
5. Prior to bone removal the DEXA study will be requested.
6. Serum samples of the same patients obtained using a polyethylene catheter will be preserved at -80°C. Serum samples may be used in a future study to establish a corelation between the bone and serum levels of trace elements.
7. A standardized A-P X-ray of both hips will be obtained to assess the Sigh index and to compare them to the results of DEXA.

In this study it is proposed to measure the trace element levels of the cancellous and cortical bones separately. The speparation of the cancelous bone from cortical bone is proposed at figure 1. The aim of such a separation may distinguish the trace element ratio between cortical thinning and loss of cancellous trabecular loss.

Trace element studies will be performed by the neutron activation analysis. Bone specimens weighing approximately 300 mg will be irradiated with thermal neutrons at Çekmece Nuclear Research Center, TR-2 reactor, in Istanbul. Gamma rays of the radioactive isotopes of the specimens will be measured with the nuclear spectroscopy system of the Department of Chemistry, METU. Atomic absorbtion spectrometry will also be used for certain elements such as Ca, Cu, Ni and few others.

2.3 Biomechanical Studies

The bones obtained and preserved by the method proposed above will be biomechanically evaluated by compression, distraction and three point bending procedures. The stress-strain relation will be evaluated for each age group. Loading and distraction rates will be standardized as 5 or 10 mm/second for each specimen. The modulus of elasticity will be calculated from the obtained data. Using the ultrasonic vibration methods, further knowledge on the mechanical structure of bones will be obtained. The Lloyd M 30 K mechanical testing device and the CNS Pondit digital ultrasonic testing machines of the Biomechanical Laboratory of the Department of Engineering Sciences will be used during these experiments.

2.4 Immunocytochemical Studies

Excluding the systematically effective parathyroid hormone, estrogen and vitamin D, some cytokines as IGF-1 (insuline like growth factor 1), TGF-β (transforming growth factor β), growth factors as IL-1 (interleukin 1), IL-4 (interleukin 4), IL-6 (interleukin 6) and mediators such as prostoglandin's. VIP (vasoactive intestinal polypeptide), SP (substance P), CGRP (calcitonin gene related peptide) and NPY (neuropeptide Y) are responsible of osteoneogenesis and bone resorption. The increase of extracellular matrix proteins as osteocalcin and bone specific alkaline phosphatase indicate bone destruction and may be a finding of osteoporosis. The evaluation of the above mentioned proteins, cytokines and local mediators by immunocytochemical means may assemble a relation between the concentrations of trace elements, biomechanical results and bone density analyses.

3. Results

Approximately 6000 patiens were evaluated by DEXA between March 1992 and November 1994. 328 of these patiens were evaluated as the normal population. The age distribution and spinal bone density analyses are presented in table 1 and figure2. These people were known to be disease free and assessment was performed due to their own request. Results of the study correlated well with the results of the normal curve of the European and American population.

Previous studies performed in the Department of Engineering Sciences, Middle East Technical University were based on the assessment of local osteoporosis induced by the implantation of calcium hydroxyapatite (CHA) ceramics [3]. Porous CHA ceramics are biocompatible and present osteoconductive properties. The radiological findings of this study revealed local porosis at adjacent sitesof the CHA ceramic. Bone marrow swelling and depletion at the implantation site were observed histologically. Osteoclasts removed particles of the implant that may be the cause of local porosis. The possible porosis was evaluated by bone densitometry analysis and compression and bending tests. CHA particles were implanted into the left limbs and a sham operation was utilised on the right limbs of 75 white rabbits. The animals were followed up for six months. The CHA implanted area and its distal and proximal adjacent areas were evaluated with a Hologic QDR-2000 (Hologic Inc., MA, USA) bone densitometer. Three point bending and compression tests were performed with an M-30 K (Lloyd Instuments, UK) material testing device. The results revealed a time dependent bone density increase at the CHA implantation site and no significant porosis at adjacent areas of the implant. The stiffness of the CHA implanted rabbit bones presented a different fracture pattern than the control group. The stiffness of the control and the CHA implanted bones generally increased with time indicating no adverse effects of porous CHA ceramics on bone and bone marrow healing.

4. Plans For Future Work

Future plans are presented in 2.2, 2.3, and 2.4, and work plan is given in Apendix.

5. References

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Table 1. Bone Mineral Density Analyses of the Turkish Population*

Age Distribution	Number	Mean ± SD	% CV
20-24	2	0.904 ± 0.013	1.5
25-29	8	0.606 ± 0.111	12.2
30-34	12	0.900 ± 0.105	11.7
35-39	29	0.975 ± 0.152	15.6
40-44	101	0.957 ± 0.120	12.6
45-49	96	0.969 ± 0.141	14.5
50-54	54	0.916 ± 0.113	12.4
55-60	30	0.848 ± 0.093	11.0

* Assesment was performed in the AP direction to the lumbar spine (L1-L4)

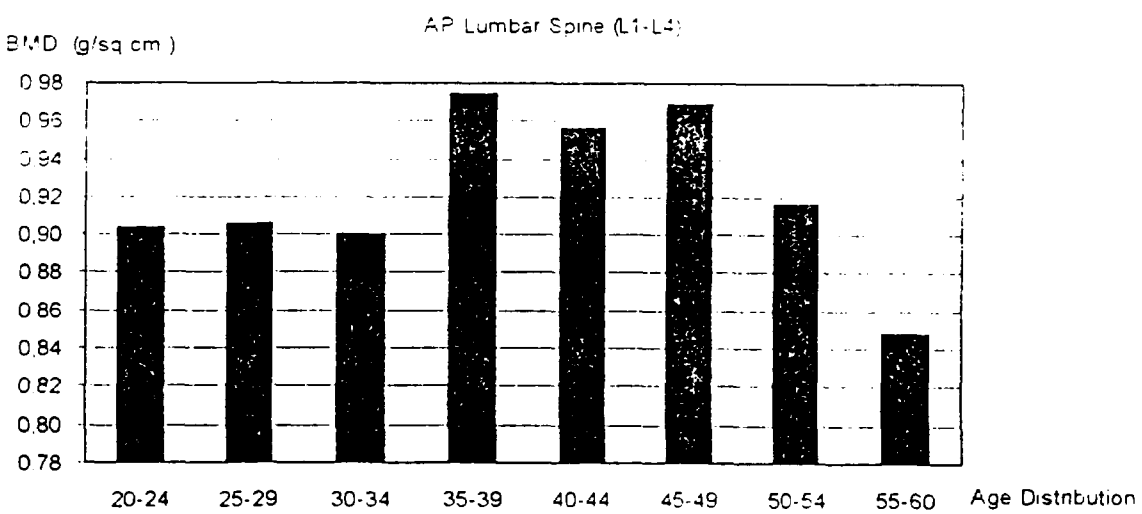

Figure 2. Bone Density Distribution as a Function of Age

6. Scientific Staff

Chief Scientific Investigator:	Professor Dr. Namık K. ARAS Department of Chemsitry. METU
Bone Density Analysis:	1. Professor Dr. Yahya Laleli 2. Levent Kuşçu MD Düzen Laboratories, Ankara 3. Feza Korkusuz MD 4. Mehmet Ungan MD Medical Center, METU
Bone Biopsy:	1. Associete Professor Uğur Günel Numune State Hospital, Ankara 2. Associete Professor Sacit Turanlı Muhittin Ülker State Hospital, Ankara
Trace Element Analyses:	1. Prof. Dr. Namık K. Aras 2. Associate. Prof. Dr. İnci Gökmen 3. Selmiye Alkan. MS Student 4. Gülay Yılmaz. MS Student Department of Chemistry. METU
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Immunocytochemical Studies:	1. Feza Korkusuz MD Medical Center. METU 2. Petek Korkusuz MD Department of Histology and Embriology Hacettepe University Medical School 3. Aykut Özkul Ph.D. Department of Virology Veterinary School of Ankara University

Osteoporoz Araştırma Formu

Adı: _____ Soyadı: _____
Adres:_____
_____ Tel _____
Doğum Tarihi:__/__/____ (gün/ay/yıl) Bayan ( ) Erkek ( )
Vücut Ağırlığı: ___ Kg Boy: ____ cm
Mesleğiniz: _____
Geçirdiğiniz Önemli Hastalık: _____
Geçirdiğiniz Ameliyat: _____

Sadece Bayanlar Tarafından Doldurulacak
İlk Adet Görme Yaşınız: ____
Adet Düzensizliği Varmı? Evet ( ) Hayır ( )
Adet Bitim Yaşınız: ____

Alışkanlıklarınız
Sıgara: Evet ( ) Günde kaç adet: ____ Hayır ( )
Alkol: Evet ( ) Günde ne kadar: ____ Hayır ( )
İlaç Kullanıyormusunuz?
Evet ( ) Hayır ( )
Cevabınız Evet ise hangi ilaçları kullanıyorsunuz?
1. _____
2. _____
3. _____
4. _____

Diyet
Günlük süt ve süt ürünü tüketim miktarınız?
Süt: _____ Peynir: _____ Yoğurt: _____
Diğer: _____

Egzersiz
Düzenli egzersiz yapıyormusunuz? Evet ( ) Hayır ( )
Cevabınız Evet ise haftanın kaç günü ve ne kadar süreyle egzersiz yapıyorsunuz?
Gün ___ Saat ___

Form for Osteoporosis

Family Name: _____ First Name: _____
Address:

_____

_____

Phone: (___) _____
Date of Birth:__/__/____ (day/month/year) Female ( ) Male ( )
Weight: ___ Kg Height: ____ cm
Occupation: _____

Previous Disease or Surgery:

_____

For Females Only
Age of Onset of Menarche: ____
Age of End of Menarche: ____

Smoking: Yes ( ) How many a Day? ____ No ( )
Alcohol: Yes ( ) How much a Day? ____ No ( )
Any Medication? Yes ( ) No ( )
If yes, please list the medicals you use:
1. _____
2. _____
3. _____
4. _____

Diet:
Daily intake of milk and milk based food?
Milk: _____(L) Cheese: _____(g)Yoghurt: _____(g)
Others: _____

Exercise:
Regular Exercise? Yes ( ) No ( )
If yes, please indicate how many days a week and for how many hours during a day you perform the exercise:
Specification of the Type of Exercise: _____
Days/ Week: _____ Minutes/Day: _____

Work Plan

Months Study	1-3	3-9	9-12	12-18	18-24	24-36
Bone Density Analysis	Evaluation of already collected data at the Düzen Laboratory	New bone density measure-ments	New bone density measure-ments	New bone density measure-ments	Analysis of data	Analysis of data, Publication
Trace Element Studies	Collection of bone samples	Collection of bone samples	INAA and ASS	INAA and ASS	Analysis of data	Analysis of data, Publication
Biomech anical Studies	Collection of bone samples	Collection of bone samples	Biomech-anical studies	Biomech-anical studies	Analysis of data	Analysis of data, Publication
Immuno-cytoche mical Studies	Collection of bone samples	Collection of bone samples	Tissue culture studies	Tissue culture studies	Analysis of data	Analysis of data, Publication

Protocol of Graft Removal

1. The iliac bone should be tricortical.
2. The size should be approximately 3.0 x 1.5 x 1.5 cm.
3. During removal a pure titanium saw will be used, and no other metal will touch the bone to prevent metal contamination. The soft tissues and blood cells will be cleaned using pure titanium instruments and de-ionised water. Prior the trace element studies the bones will be treated with carbon tetra chloride and defatted by methanol.
4. The bones will be preserved in polyethylene bags at -20°C. The inside of the bags will be rinsed with de-ionized water.
5. Prior to bone removal the DEXA study will be requested.
6. Serum samples of the same patients obtained using a polyethylene catheter will be preserved at -80°C. Serum samples may be used in a future study to establish a corelation between the bone and serum levels of trace elements.
7. A standardized A-P X-ray of both hips will be obtained to assess the Sigh index and to compare them to the results of DEXA.

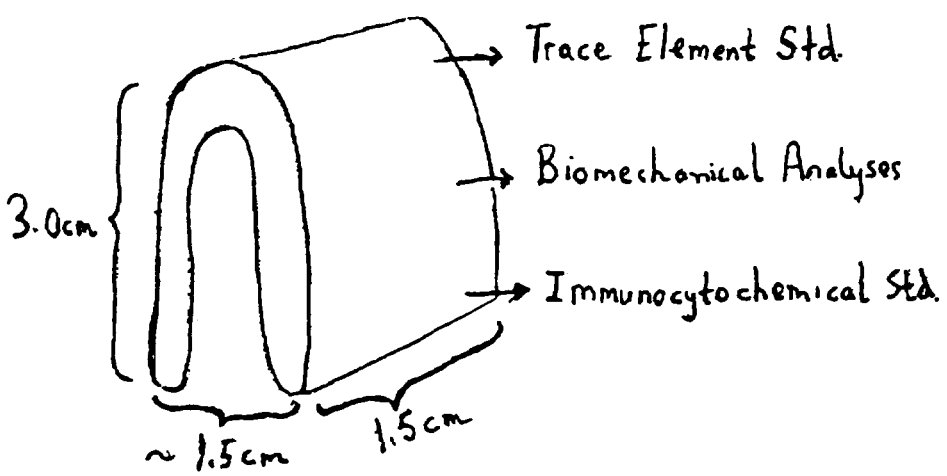

Figure 1