SCREENING POST-MENOPAUSAL WOMEN FOR BONE MINERAL LEVEL BY BIOELECTRICAL IMPEDANCE SPECTROSCOPY OF DOMINANT ARM

by

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ABSTRACT

SCREENING POST-MENOPAUSAL WOMEN FOR BONE MINERAL LEVEL BY BIOELECTRICAL IMPEDANCE SPECTROSCOPY OF DOMINANT ARM

Dominant arm bioimpedance spectroscopy (BIS) and lumbar and hip dual energy x-ray absorptiometry (DXA) measurements were conducted simultaneously on 48 post - menopausal women, aged between 43 and 86 years, with no hip or arm fracture history at Department of Radiology of Istanbul University Cerrahpaşa Hospital. According to lumbar DXA results, 21 women were classified as normal, 22 as osteopenia and 5 as osteoporosis; whereas hip DXA results classified 30 women as normal, 15 as osteopenia and 3 as osteoporosis. Only 26 participants had identical lumbar and hip BMD diagnostic results. Dominant arm characteristic frequencies of normal subjects were significantly different from osteoporotic subjects based on both lumbar ($p < 0.005$) and hip classification groups ($p < 0.001$). Hip and lumbar spine DXA BMD values were significantly correlated ($r = 0.55$, $p < 0.005$). The dominant arm BIS characteristic frequency, considered as the one of the single predictors in earlier diagnosis of osteoporosis, was found negatively correlated with DXA measurements for both hip and lumbar spine regions. The Spearman rank correlation coefficient of BIS values with the hip DXA values ($r = -0.53$, $p < 0.001$) was higher than that of lumbar spine ($r = -0.37$, $p < 0.001$). In receiver operating characteristic (ROC) curve analysis, the best discrimination of dominant arm characteristic frequency was made between normal and osteoporotic subjects based on the hip subgroups ($p < 0.001$). Both lumbar bone mineral content (BMC) ($r = -0.47$, $p < 0.001$) and hip BMC ($r = -0.4340$, $p < 0.005$) were significantly correlated with dominant arm characteristic frequency.

Keywords: bioimpedance spectroscopy, bone mineral content, osteoporosis, osteopenia, bone mineral density, dual energy x - ray absorptiometry.

ÖZET

MENAPOZ SONRASI KADINLARDA KEMİK MİNERAL SEVİYESİNİN BASKIN KOL BİYOELEKTRİK EMPEDANS SPEKTROSKOPİ İLE TARANMASI

İstanbul Üniversitesi Cerrahpaşa Tıp Fakültesi Radyoloji bölümünde, geçmişte kalça veya kol kırığı bulunmayan 43 - 86 yaşları arasında 48 menapoz sonrası katılımcıya sırası ile sırt ve kalça dual enerji x - ray absorpsiyometri (DXA) ve baskın kol biyoempedans spektroskopi (BIS) yöntemleri uygulanmıştır. Sırt bölgesinden alınan DXA sonuçlarına göre katılımcılar gruplandırıldığında, 21' i normal, 22'si osteopeni ve 5'i osteoporoz olarak belirlenmiştir. Gruplandırma kalça bölgesi DXA sonuçlarına göre yapıldığında ise, 30 hasta normal, 15 hasta osteopeni ve 3 hasta osteoporoz olarak belirlenmiştir. Katılımcıların 26' sı hem sırt hem de kalça bölgesine göre aynı grupta yer almıştır. Hem sırt ($p < 0.005$) hem de kalça ($p < 0.001$) DXA sonuçlarına göre oluşturulan sınıflandırmalarda normal ve osteoporotik olarak belirlenmiş katılımcıların baskın kol karakteristik frekans değerleri istatistiksel olarak anlamlı bir şekilde farklı çıkmıştır. Kalça ve sırt DXA sonuçları birbiriyle anlamlı korrele bulunmuştur ($r = 0.55$, $p < 0.005$). Erken menapozun önemli bir belirteci olarak görülen BIS baskın kol karakteristik frekansı, hem kalça hem de sırt DXA kemik mineral yoğunluğu ile ters korrele bulunmuştur. BIS karakteristik frekansının kalça bölgesi DXA değerleri ile Spearman sıra korrelasyon katsayısının ($r = -0.53$, $p < 0.001$) sırt bölgesi DXA değerleri ile olan korrelasyon katsayısndan ($r = -0.37$, $p < 0.001$) daha yüksek olduğu görülmüştür. ROC eğrileri incelendiğinde, DXA ile kalça bölgesi sonuçlarına göre oluşturulan gruplarda normal ve osteoporotik katılımcıların en iyi şekilde ayrılabildiği görülmüştür ($p < 0.001$). Hem sırt ($r = -0.47$, $p < 0.001$) hem de kalça ($r = -0.4340$, $p < 0.005$) kemik mineral içeriği (BMC) baskın kol karakteristik frekansı ile korrele bulunmuştur.

Anahtar Sözcükler: biyoempedans spektroskopi, dual enerji x–ray absorbsiyometri, kemik mineral içeriği, kemik mineral yoğunluğu, osteoporoz, osteopeni.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS

A	Cross - Sectional Area
α	Significance Level
ρ	Density
L	Length
P	Significance Level
R	Resistance
Xc	Reactance
V	Volume
Z	The Total Impedance

LIST OF ABBREVIATIONS

BI	Bioelectrical Impedances
AUC	Area Under Curve
BIA	Bioelectrical Impedance Analysis
BIS	Bioelectrical Impedance Spectroscopy
BMC	Bone Mineral Content
BMD	Bone Mineral Density
BMI	Body Mass Index
CI	Confidence Interval
CT	Computed Tomography
DXA	Dual Energy X-ray Absorptiometry
ECW	Extracellular Water
FDA	U.S. Food and Drug Administration
FM	Fat Mass
ICW	Intracellular Water
LBM	Lean Body Mass
MF	Multi Frequency
MM	Muscle Mass
MRI	Magnetic Resonance Imaging
NIH	National Institute of Health
NS	Not Significant
QCT	Quantitative Computed Tomography
QUS	Quantitative ultrasound
ROC	Reciever Operating Characteristics
SF	Single Frequency
SXA	Single Energy X - ray Absorptiometry
TBW	Total Body Water

1. INTRODUCTION

Bone strength is a factor that notably affects the daily life of a person, where decreased bone strength could cause challenges such as hip fractures and fragilities [1, 2]. Osteoporosis is defined as the decrease in bone strength and bone mineral density (BMD) and occurs with ageing (senile osteoporosis) or during the post-menopause period [3, 4]. The probability of osteoporosis occurrence is higher if the body did not reach its maximum bone density at early ages [5, 6].

Post-menopausal osteoporosis is the loss of trabecular bone after menopause with changes in body composition and hormones [7]. The proportions of fat, muscle, and lean body mass in the body vary with estrogen deficiency [7]. In a recent study, vitamin D level and BMD in women during the menopausal and post - menopausal period were found to be strongly correlated [8].

Osteoporosis progresses silently and its symptoms are not always visible [9]. Several methods have been proposed for estimating BMD, including radiographic absorptiometry, quantitative ultrasound (QUS) , quantitative computed tomography (QCT), magnetic resonance imaging (MRI), computed tomography (CT), single energy X-ray absorptiometry (SXA) and dual energy X-ray absorptiometry (DXA). Despite the vast amount of the information they provide, radiation dose and contrast agent intake are some of the drawbacks of the CT and MRI methods, respectively. Moreover, both systems are expensive and the preparation process is long [10, 11, 12]. Bone mineral density is usually measured with DXA and SXA in peripheral regions.

Dual X-ray absorptiometry (DXA) is the current gold-standard method for assessing BMD. DXA measures the bone mineral density of total body through hip or lumbar spine. The forecast of hip fracture is performed effectively by measuring both hip and lumbar spine regions; as hips are the most effective regions for fracture

comparisons [10, 11, 12]. Body bioimpedance characteristics are a function of body composition as well as bone mineral content. With foot to foot, single frequency (50 kHz, 0.8 mA) bioimpedance measurements of the whole body in postmenopausal women (42 - 84 years) and men (42 - 94 years), it was shown that the bioimpedance was correlated to the BMD, with a relatively higher correlation in men [13]. In a recent study, Cole characteristics of complex electrical impedance measurements from different body compartments in post-menopausal women were compared against their reference DXA bone mineral density classifications and the characteristic frequency, i.e. the frequency at which the impedance phase shift is maximized in magnitude, was shown to have the strongest correlation with BMD, for the dominant arm [14, 15].

Osteoporosis is very commonly encountered in post-menopausal women. There is an increased need for a low cost and efficient screening alternative to address this population. In this study, a segmental bioimpedance spectroscopy of only the dominant arm and DXA measurements of lumbar and hip regions were collected simultaneously on post-menopausal women to investigate the usability of bioimpedance analysis (BIA) as a screening tool in bone mineral density assessment.

2. BACKGROUND OF THE STUDY

2.1 Bone Structure

Bone tissue contains two types, cortical and trabecular bone. Each bone contains these two types bone tissues, but the quantities and the regions in which they are located are different. Cortical bone is also called compact bone. It forms the outside of the bone. Its is highly dense. Trabecular bone is also called cancellous bone or spongy bone. It has less density than the cortical bone. It is more porous than cortical bone. Skeleton is constructed by 20 percent trabecular bone and 80 percent cortical bone. Cortical bone dominates the shaft of the long bones and femoral neck. Cortical bone compromises the majority of human skeletal. Generally, it is the surface layer of the bone and it is stronger to protect. Trabecular bone is porous and it covers marrow and blood vessels inside of the pores. Moreover, the regeneration of trabecular bone tissue is faster than cortical bones. The capability of regeneration decelerates with increased age. This reduction could cause osteopenia and osteoporosis (low bone mineral density) [16, 17].

Bone tissue is steadily remodeled. Bone degeneration and remodeling is done by osteoclasts and osteoblasts, respectively. These are the main responsible cells of the bone remodeling cycle. How bone remodeling cycle starts is unknown. However, it is assumed that the aim is to defaecate damaged bone areas [17].

2.2 Osteoporosis

Albright, for the first time in the 1950s, found that osteoporosis was a form of bone destruction and this was the first time that he used this name. He has done research on women who are postmenopausal and young premenopausal [18], [5]. Then in 1988, osteoporosis was defined by National Institute of Health (NIH) [5].

According to NIH, bone quality and bone density are two essential characteristics of a bone. Bone density is related to mineral amount. Bone quality is related to the structure and shape of the bone. Osteoporosis is a bone structure disease caused by the change of bone strength. Osteoporosis is not just a bone loss disease. Bone loss is seen after middle age. However, the rate of bone loss is important. Maximum bone density reached in young or middle age is also important for osteoporosis. The probability of osteoporosis occurrence is higher if the body did not reach its maximum bone density at early ages [5, 6].

Osteoporosis is the bone mineral density (BMD) deficiency. It weakens bones and bones become fragile and open to fracture easily [16]. Two types of osteoporosis exist: postmenopausal and age related osteoporosis. Age related osteoporosis occurs in both men and women. It grows slowly with ageing. Postmenopausal osteoporosis grows rapidly after menopause [16]. Post-menopausal osteoporosis is the loss of trabecular bone after menopause with changes in body composition and hormones [7]. The proportions of fat, muscle, and lean body mass in the body vary with oestrogen deficiency [7]. Osteoporosis progresses silently and its symptoms are not always visible [9, 19].

For decades, osteoporosis was diagnosed with backpain syndrome, vertebral fractures and osteopenia on plain films. Generally, the objective of clinicians is to diagnose the secondary causes of low bone mass. However, it has become a primary disorder in the last decade [16]. Osteoporosis is more prevalent in post-menopausal women than pre-menopausal women and men.

Osteoporosis is a quite common health problem. 22 million women and 5.5 million men had it in EU in 2010. Moreover, 3.5 million new fragility fractures existed, including 620.000 hip, 520.000 vertebral, 560.000 forearm and 1.800.000 other fractures. The cost of incident and prior fragility fractures was estimated at 37 billion euro. These amounts are expected to increase by 25 % by 2025 [20, 21].

2.2.1 Factors Affecting Osteoporosis

When examining the change in bone density in women, it is necessary to consider other physical and genetic factors as well. There are a lot of factors affecting the risk of osteoporosis and the decreasing of bone density in women, some of which are mentioned below.

Bone mass reaches its maximum value by the age of 35. Then it starts to decrease slowly every year. This reduction accelerates after menopause in women. Genetic factors influence bone quality by more than 50 percent. Bone quality, lifestyle and environmental factors in younger ages also affect the rate of bone density decline in years. Some of these factors are alcohol consumption, smoking, dietary and exercise habits [22, 23].

Obesity is the first factor that can affect bone quality. Obesity is the condition in which the amount of energy received is greater than the amount of energy spent. Obesity is determined by body mass index (BMI). BMI is calculated by dividing a subject's weight by square of his height. Adults with a body mass index greater than 30 kg/m^2 could be classified as obese. BMI classifications are shown in Table 2.1. The classification of obesity varies according to sex, age etc. Unstable living conditions, and irregular and excessive nutrition intake cause obesity. Obesity is becoming increasingly widespread as the changing living conditions have more sedentary lifestyle. At the same time, the spread of fast food products is also an effect that increases obesity. Obesity can cause other disease such as, high blood pressure, diabetes etc. Obesity is a common health problem. It is also a matter of curiosity how obesity affects bone structure [22, 23, 24].

Table 2.1
BMI Distribution.

Status	BMI
Normal	< 25.0
Overweight	(25.0 - 30.0)
Class 1 (low - risk) obese	30.0 - 35.0
Class 2 (moderate - risk) obese	35.0 - 40.0
Class 3 (high - risk) obese	$40.0 <$ BMI

Previous studies have shown that fat mass has positive effects on bone density. When fat mass increases bone density increases [25]. However, it is not known whether excessive amount of fat mass prevents osteoporosis [22].

Moreover, slim women are more likely to have osteoporosis, while overweight women are less likely to have it. Osteoporosis rate is decreasing in overweight women [26]. Studies also show that muscle movements and lifting exercises increase bone mass in the body [22].

Eating habits affect human body in many ways. Osteoporosis can be one of the results of insufficient food intake. Regular eating habits, regular intake of vitamins and minerals are very important for a healthy life. The nutrients taken for a healthy bone structure are also very important. Bone structure needs vitamins and minerals to protect and to regenerate itself. Nutritional supplements should be taken when deficiencies are found [23, 22, 8, 27] . The daily necessary nutrition of each person should be determined according to age, gender, and physical activity [24].

According to studies, excessive alcohol consumption negatively affects bone density over time and increases the risk of osteoporosis. Excessive alcohol consumption, especially at young ages, increases the likelihood of osteoporosis in the following years.

It was observed that the bone structure of subjects with excessive alcohol consumption had less mineral and water. Moreover, in studies, even if the subjects stopped drinking alcohol after a while, it was observed that the regeneration of bone structure did not increase. Results are supported by animal experiments [22]. Excessive alcohol consumption and reduced bone density experiments in adult women and animals also support the negative impact of alcohol consumption on bone density. According to Hannan and Hogan, subjects with excessive alcohol consumption experience bone loss faster than those consuming less or none [28, 29].

The effect of moderate alcohol consumption on bone density is not fully understood. Some studies indicate that moderate alcohol consumption has an enhancing effect on bone density [22].

Smoking negatively affects human health in many ways such as lung cancer, high cholesterol, heart diseases, poor vision, anxiety, unhealthy teeth, early menopause etc. Also, smoking influences BMD adversely. It increases the possibility of osteoporotic fractures [27, 30]. Heavy smokers are more likely to have osteoporosis than nonsmokers. However, moderate smokers are not statistically significantly different from nonsmokers [31]. Most of nonsmokers are exposed to cigarette smoke. This may be the reason why there is no significant difference between the two groups.

2.2.2 Diagnosis of Osteoporosis

Measuring BMD is one way of osteoporosis diagnosis and BMD is a quantitative parameter. It is determined by T scores' distribution. A patient that has a T score of between -1 and 1 gets classified as normal, while a patient whose T score is less than -1 is classified as osteopenia and osteoporosis. Other parameters, called secondary parameters (age, BMI, smoking, other diseases, medicines etc.) also affect osteoporosis and hip fractures. While risk of fractures are analyzed, all factors should be considered for a correct diagnosis. However, it is not possible to observe all factors.

Currently, some of the risk factors are considered. The probability of osteoporosis is analyzed with FRAX tool. Ten year probability could be analyzed with this tool [32].

Not reaching peak bone mass and a rapid decrease in bone density in adulthood may be the signs of osteoporosis. A person that has both low peak bone mass in youth and rapid decrease in bone density, has high fracture risk. On the other hand, the possibility of fracture risk in subjects who have low peak bone mass and low bone loss is the same as the risk of the subjects that have high peak bone mass and rapid bone loss. Peak bone mass is related to the biological inheritance, so we could say that osteoporosis and fracture risk are associated with genetics. Thus, subjects who have osteoporosis history in their family, should get their bone levels checked regularly [5].

The region of where the DXA measurement is taken plays a key role in determining osteoporosis. Hip BMD region is better to determine fracture risks than lumbar spine. Also, after 65 years old, DXA measurements should be taken from femur/hip region in order to eliminate the effects of osteoarthritis [33].

Getting BMD measurements is suggested in women after 65 years old or postmenopausal women [32, 34]. Only BMD measurements are not sufficient to diagnose osteoporosis and osteoporotic fractures in premenopausal women and men aged 50 years old or below. Secondary factors should be included in diagnosis [33].

2.3 Body Compositions and Bone Mineral Density

Body compositions are mainly total body water (TBW), intracellular water (ICW), extracellular water (ECW), , fat mass (FM), muscle mass (MM), fat free mass (FFM). All body compositions have different characteristics and they are composed of different components. These components affect the properties such as the permeability and resistance of the body compositions. Conductivity increases as the amount of water in the body composition increases. Muscle mass and fat free mass has higher

conductivity than fat mass. Fat mass has the highest resistance between them because it has less water. Body compositions are analyzed generally in two groups; lean body mass (LBM) and fat mass (FM). Lean body mass consists of TBW (which is consist of ECW and ICW) and bone tissue.

2.3.1 Body Compositions and Bone Mineral Density Analysis Methods

There are a number of methods for examining body compositions such as total body composition (TBW) measurements, hydrodensitometry, anthropometry, neutron activation, computed tomography, magnetic resonance imaging, BIA, and dual energy x - ray absorptiometry [35]. Anthrometry is a method that gathers information of body size, compositions and structure. The main parameters of anthropometry are weight and height. Hydrodensitometry measures body compositions in the water. It is also called underwater weighing. Body density is measured by using water and body density differences. A tank with full of water and a chair hanging in the tank are used for this measurement. Total mass is measured both with and without the subject. Also, subject's weight is measured at the outside of the tank. Fat percentage of the subject is calculated with special equations after finding body density. This method may underestimate or overestimate, because it assumes bone and body fat percentages. Hyrodensitometry, anthropometry and neutron activation are whole body measurements, which are not enough for regional measurements. Regional body composition analysis could be done by CT, MRI and DXA [35].

Body component analysis could be conducted in four different dimensions. The first is the measurement of atomic dimensions such as calcium, potassium and hydrogen. It is measured by direct methods such as total body counting and neutron activation. The second measurement is in the molecular dimension such as water and oil. The third could be measured in tissue dimensions such as muscle and skeleton. The forth is cellular dimensions, such as intracellular and extracellular water. The methods used for these are different. BIA and anthropometry are indirect methods

and they make predictions. The tissue characterization is done by CT, MRI and DXA measurement techniques. They are called criterion methods.

In order to diagnose osteoporosis, BMD measurements are used. Several methods have been proposed for estimating BMD, including radiographic absorptiometry, single energy X - ray absorptiometry (SXA), dual energy X - ray absorptiometry (DXA), quantitative computed tomography (QCT), computed tomography (CT), quantitative ultrasound (QUS) and magnetic resonance imaging (MRI). Despite the vast amount of information that they provide, radiation dose and contrast agent intake are some of the drawbacks of the CT and MRI methods, respectively. Moreover, both systems are expensive and the preparation process is long [10, 12, 11]. BMD is usually measured with DXA and SXA in peripheral regions. The gold-standard method to assess BMD is DXA. DXA measures the bone mineral density of total body through hip or lumbar spine. The forecast of hip fracture is performed effectively by measuring both hip and lumbar spine regions; as hips are the most effective regions for fracture comparisons [10, 12, 11].

QCT first appeared in the mid-1970s. It is used along with CT scanners [36]. Real volumetric BMD can be assessed by QCT. It is not size dependent, so it eliminates physical measurement mistakes. Also, trabecular and cortical bone can be differentiated. However, the system has some drawbacks such as high price and higher radiation dose exposure [32]. Moreover, it is not widely used as DXA. The subjects are exposed to more radiation when scanning with QCT than DXA. High radiation exposure is one of the reasons why QCT is not preferable as much as DXA. On the behalf of CT technology improvements, QCT has many clinical advantages. 3D volume images can be taken rapidly by CT. It can serve more detailed information about both cortical and trabecular bone [39]. QCT is inserted in a CT device, so subjects are examined with CT scan for other diagnosis and bone mineral density is estimated by using the same system. Operators may not be prefer QCT for osteoporosis because of large workload in crowded hospitals.

Quantitative ultrasound has many benefits including low cost, no radiation dose, and being easily accessible. The principle of this technique is reflection of ultrasound waves from the bone [32]. Also, QUS gives dimensional information about bone. This information is useful to understand bone quality along with BMD [37]. Contact and water bath are the two methods of QUS. Contact is approved only for initial screening, while water bath is approved for both initial screening and follow up scans by U.S. Food and Drug Administration (FDA) [37]. QUS is an alternative predictive method to diagnose osteoporosis and fractures. However, QUS does not provide detailed information, so it may not be enough for routine diagnosis. QUS may be used as an initial scanning method to understand which patients should undergo BMD measurements [37].

2.3.2 Dual Energy X - Ray Absorptiometry (DXA)

Recently, DXA is the most widely used and the most reliable system for bone mineral density measurements. DXA is used to measure bone mineral density, bone mineral content and bone mineral compositions. BMD can be measured in many sites of the body by using DXA. However, most preferable sites are lumbar spine and hip [33]. The DXA measurements are made by utilizing the attenuation of the tissues in two different X - Ray energies [38, 39]. DXA technique is used to recognize osteoporosis or osteopenia. Changes in bone structure over time could be observed with this method. The results of bone mineral content and bone mineral density taken from femur and spine are shown on the DXA monitor as can be seen in Figure 2.1 and Figure 2.2. The information of patients' age, sex, height, weight, BMC, BMD, T and Z scores could be monitored at the screen. When it is needed, these information could be printed and stored [35]. DXA has many advantages (simple, rapid and exposing less radiation), while it has some restrictions, including ineffective discrimination of different bone tissues and having only area measurement [39].

Specific standards for DXA results have been established by evaluating the

results from young healthy individuals. The patient outcomes from the DXA are compared with these standards and the deviations from the normal are calculated to diagnose osteoporosis [39]. BMD acquired from DXA is analyzed according to difference from the mean of the reference population. T scores are calculated based on the deviation from reference population's mean, and are used to determine bone status, such as normal, osteopenia and osteoporosis. The distribution of T scores could be seen in Figure 2.3 and Table 2.2. While T scores higher than -1 are classified as normal, T scores less than -1 indicates that the subject has low bone density (osteopenia) or osteoporosis [3].

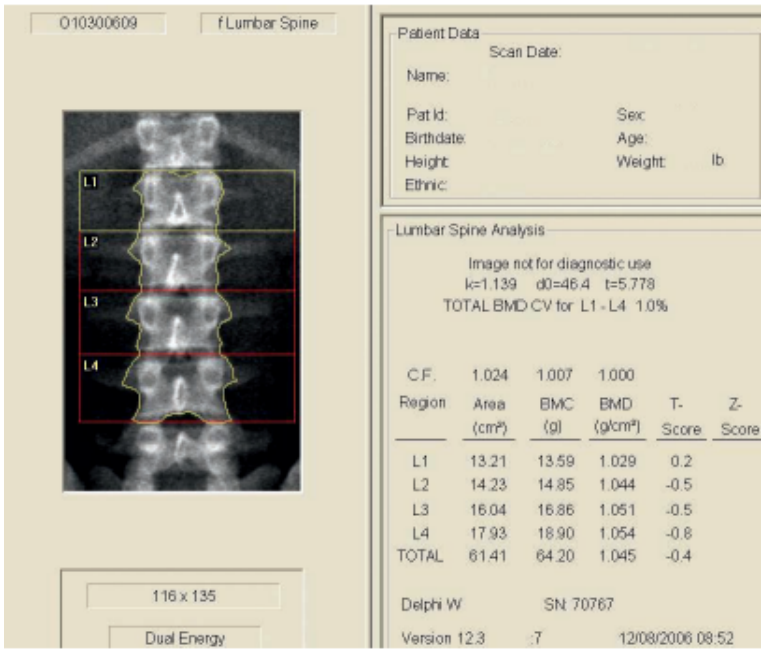

Figure 2.1 Lumbar Spine DXA Scan [35].

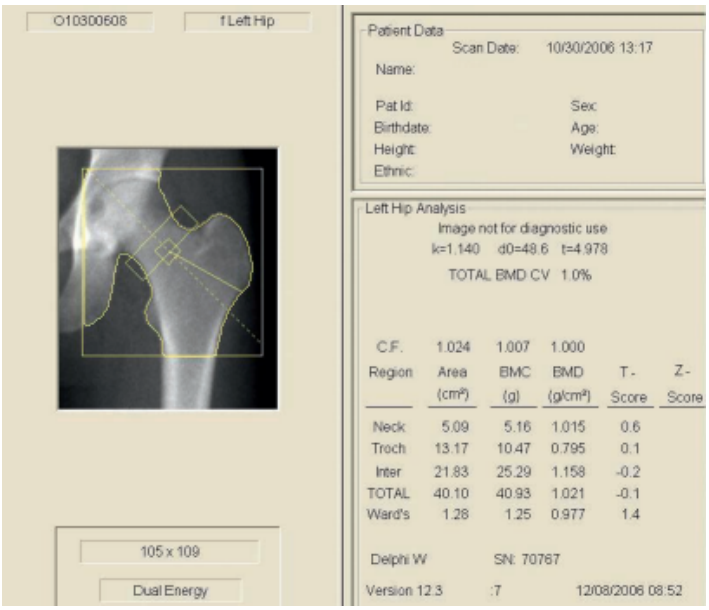

Figure 2.2 Femur Hip DXA Scan [35].

Table 2.2
T score distribution.

Status	T score
Normal	$>$ -1.0
Osteopenia	(-2.5. - -1.0)
Osteoporosis	$<$ -2.5

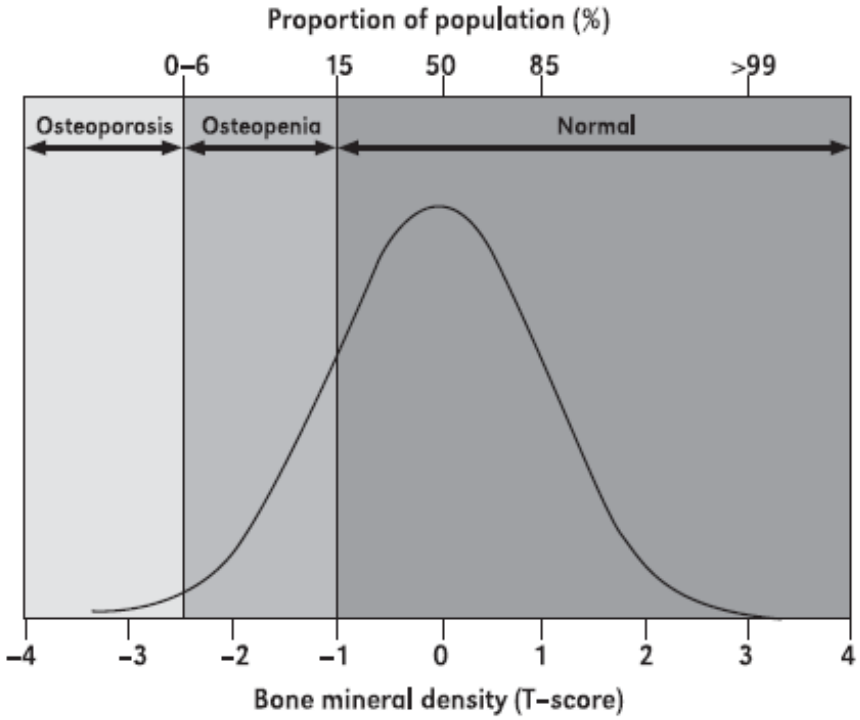

Figure 2.3 T score Distribution [40].

Z scores are also a way of analyzing BMD. Unlike T score, age, origin, gender are also used to define Z score. Z scores are determined according to normal distribution in the same age group. Standard deviations are also used to classify bone status [40]. The sample of BMD, BMC, T score and Z score results of a DXA scan is shown in Figure 2.4.

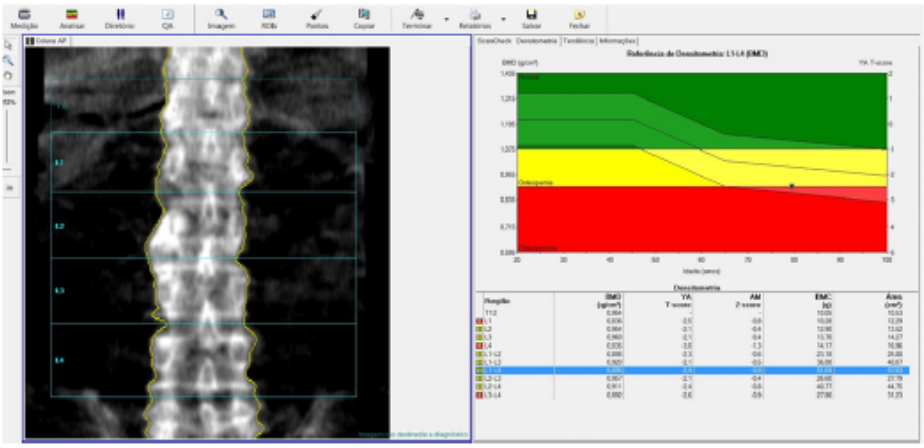

Figure 2.4 BMD, BMC, T and Z scores [41].

Generally, DXA scans start with lumbar spine. Then one of the hips are scanned. Positioning should be correct during both scans. Positioning is manually controlled by the technicians [42]. For a correct diagnosis, a single vertebra scan should not be used. If there is noise in one of the scans, forearm DXA is performed [42]. The best diagnosis could be made with lumbar spine scan BMD taken from four vertebrea (L1-L4) as BMD increases from L1 to L4 [42].

2.3.3 Bioelectrical Impedance Analysis (BIA)

According to the results of an NIH conference in 1994, BI is analysed by a single frequency (SF) method at 50 kHz. SF-BIA was a common method in 1994 [46]. However, it had some limitations. Some rules were approved to overcome these limitations. Some of these rules were about distribution of body liquids, body shape and penetration of frequency [43]. Moreover, BIA was not considered as a practical method for body compositions [44]. After, NIH conference in 1994, BIA was improved. A parallel resistance was inserted to the system to improve BIA. The other improvement method was multiple frequency BIA (MF-BIA). The aim was to enhance the discrimination of body waters. MF-BIA first appeared as two frequencies (high and low frequency). Later, more frequencies were used to measure body impedances [44].

BIA is a widely used method for measuring body components, because it is easy to measure, economical and fast. It is generally used in hospitals, sports halls, dietician's offices. It provides information about the body components of the patients during the examination. Thus, it is easy to apply special treatment to each patient. Fat free mass, fat mass and total body water measurements could be performed with BIA devices [45].

According to the working principle of the BIA, the device sends a certain amount of current to the body, leading between the two electrodes, called the current source and detector. According to Ohm's law, a voltage occurs between these two electrodes. Resistance to electric current passing through the body forms the basis of bioimpedance measurements. Body is assumed as an uniform cylinder which can be seen in Figure 2.5. Current passes through the length and passes perpendicularly to the cross-sectional area of the cylinder. This assumption enables an easy measurement of resistance of the body [43, 45, 46].

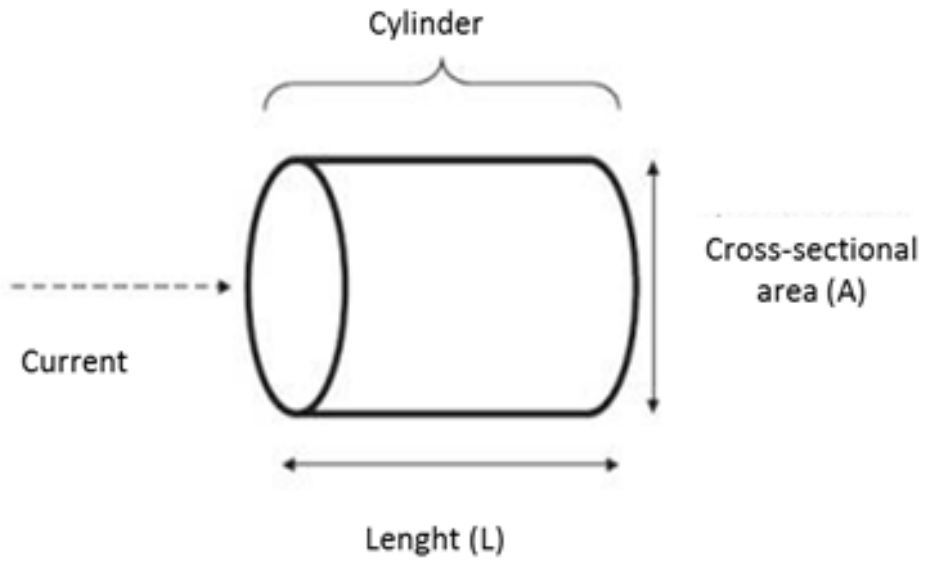

Figure 2.5 The Principles of Body Impedance Analysis [46].

The current passes through the cylinder which is assumed to be homogeneous. The resistance of the cylinder to the current is related to the length of the cylinder,

the cross-sectional area and the applied frequency. The resistance of a homogeneous conductive cylinder is proportional to its length (L) and inversely proportional to its cross-sectional area. Human body could be modeled like this cylinder, even if it is not uniform and the conductivity of body varies. The relationship between length, cross sectional area, specific resistivity (ρ), the magnitude of resistance (R) and volume (V) is described by [43, 45, 46],

$$V = \frac{\rho(L^2)}{R} \tag{2.1}$$

Lean muscle tissue, fat and bone are different biological structures, and they have different resistances against current. For example, while lean muscle tissue is conductive, fat and bone are non-conductive. The current would pass more through extracellular fluids and lean muscle tissue as it prefers to go through less resistant tissues [43, 45, 46, 47].

Human body is considered to be composed of 5 different cylinders which are arms, torso and legs. These cylinders are illustrated in Figure 2.6. Bio-impedances are analyzed by using these cylinders. The measurement could be taken from only one cylinder (segmental) or combination of all cylinders (whole body). The segment of the measurement is determined by the placement of electrodes. [43].

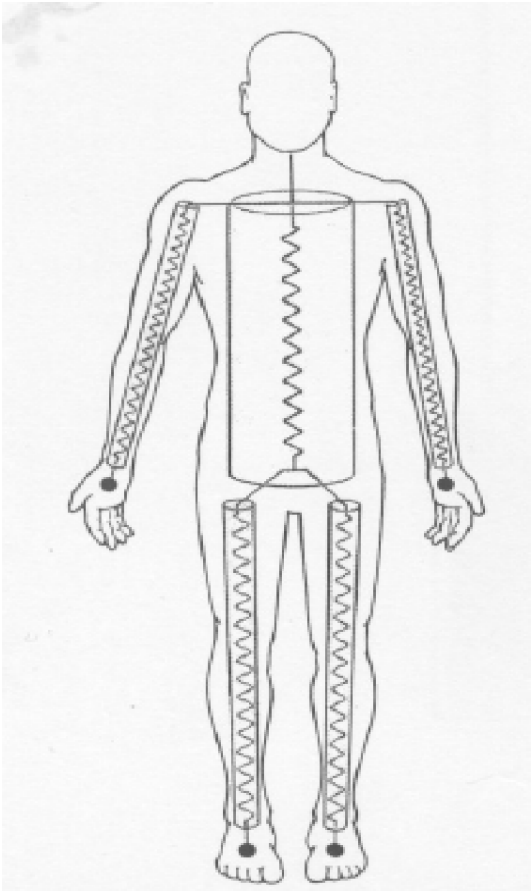

Figure 2.6 Body Impedance Model [43].

There are two components of impedance, resistive (resistance, R) and capacitative (reactance, X_c). Reactance occurs because of capacitance originated by the cell membrane and tissue interfaces, while resistance occurs from the flow of electric current through tissue fluids [43, 45]. The function of impedance is given by,

$$Z^2 = R^2 + X_c^2 \tag{2.2}$$

$$Z = \rho(L/A) \tag{2.3}$$

Resistance and reactance joins in parallel or in series. R_{zero} is measured when

the current passed through extracellular space because at low frequency, cell membrane runs as an insulator. Contrast to R_{zero}, R_{inf} is measured at high frequencies because membrane acts as an excellent capacitor (Figure 2.7). [43, 45].

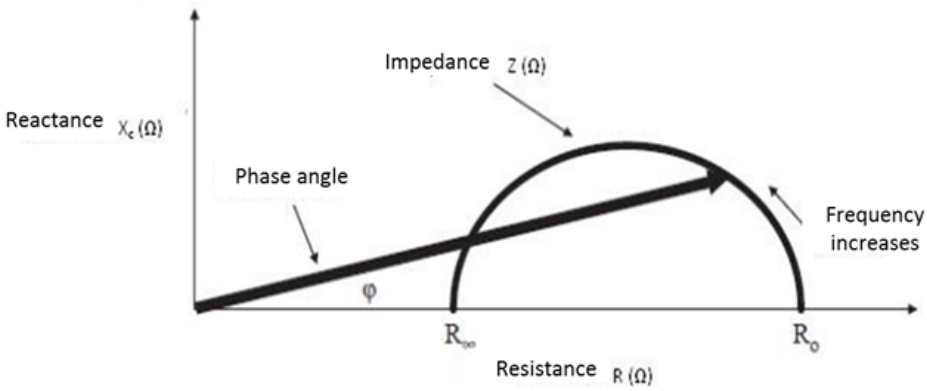

Figure 2.7 Relationship Between Phase Angle, Resistance, Reactance, Impedance, and Applied Current Frequency [45].

When human body is assumed consisting of five different cylinders, segmental BIA could be applied. To apply segmental BIA, electrode placements should be changed for each measurement. In BIA, 4 different type of electrodes are used to gather signal from the body. These are current carrying, measuring, pick up and both current carrying and measuring electrodes. The placement of electrodes differs according to how many electrodes are used to measure BIA. When two electrodes are used, electrodes should be both current carrying and measuring. When three electrodes are used, one electrode is current carrying, the other one is measuring and the last one is signal pick up electrode. If the system has four electrodes, two of them are signal pick up and others are current carrying electrodes. Electrode placements and the distance between adjacent electrodes influence the results of BIA [48].

2.3.4 Bioelectrical Impedance Spectroscopy (BIS)

BIS technique follows the same principles as BIA. Moreover, BIS technique is based on mathematical modeling and uses the Cole - Cole plot (Figure 2.7) and Hanai formula. Using these formulas, R_{zero} and R_{inf} are found by using the link between R

and body fluid cells. In this technique, healthy young subjects are used to estimate R_{zero} and R_{inf} [43, 45].

BIS measurements are obtained by inserting the current source and the detecting electrodes in the body according to the region to be measured. The BIS technique offers alternatives in terms of regions where the electrodes are located. For example, in Figure 2.8, current source and pick up electrodes are inserted at right arm and right foot. In addition to the hand-to-foot technique used in standard methods, foot-to-foot and hand-to-hand electrode placement could be used. The fact that the electrodes could be placed in different zones makes the BIS technique more advantageous than standard methods [45, 46, 49].

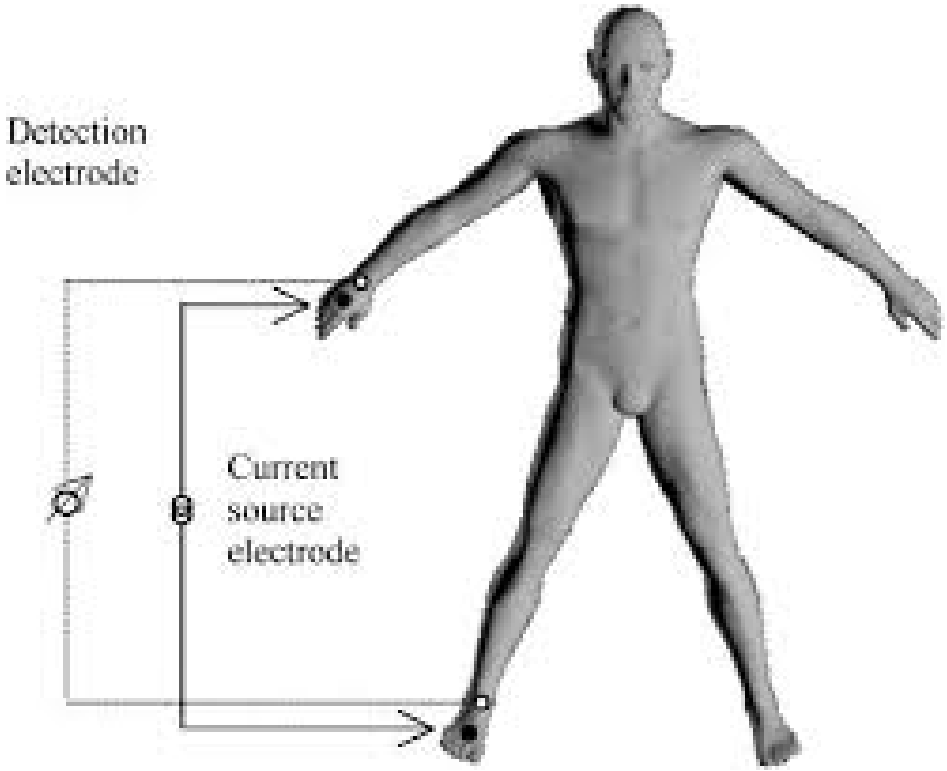

Figure 2.8 Electrode Placements [43].

The Factors That Affect BIA

Many factors exist that affects bioimpedance analysis. These factors influence each method (SF-BIA, MF-BIA and BIS) differently. Single-frequency BIA has some disadvantages in comparison to multi-frequency and segmental BIA. Multi-frequency and spectroscopy methods have benefits from acquiring multi-frequency data that enables to find optimal mean value. On the other hand, single-frequency methods are measured in single frequency as the name implies and it restricts the analysis [45].

Food and beverage intake influences the results of BIA. Impedance may be reduced by eating food or drinking before analysis. Also, eating could cause slight miscalculations. To provide accurate measurements, subjects should not eat or drink anything just before the measurements [45].

The results of BIA are also influenced by body positions. Measurements taken by sitting, standing or lying positions may differ from each other. Standing upright or hunching could also affect the results. The distance between adjacent peripherals or the distance of arm from torso are crucial factors. Subjects' position should be same at each measurement and they should not interact with any metal surface. Being connected to a metal surface or having metal accessories also affect the measurements [45].

Physical activity affects body composition distribution. Reduction in impedance may occur after physical activity. Measurements should not be taken after exercise. At least an hour is needed after training. Fast walking just before the measurement could also affect the results, so subjects should be rested for a while before the examination [45].

Human body temperature is affected by ambient air temperature, especially peripheric areas, foot and hand. When skin temperature varies, the results of BIA may change. Variance in ambient air may influence bioimpedance analysis. Resistance

in 15 C degree was measured to be higher than in 35 C degree. However, significant differences are not observed in slight temperature variations (between 20 - 25 C degree) [50, 51].

To sum up, the amount of fluid taken recently, body position, physical activities, ambient conditions (air) and body temperature may affect BIA measurements. For this reason, it is important to keep the conditions as same as possible when applying BIA.

3. MATERIAL AND METHODS

3.1 Data Collection and Analysis

This research was conducted, in accordance with the Helsinki Declaration, at Department of Radiology of Istanbul University Cerrahpasa Hospital and was approved by their Ethical Committee of Clinical Research. Informed consent was obtained from all participants before starting this study.

48 post-menopausal women, aged between 43 - 86 years, with no hip or arm fracture history, participated in the study. All subjects were DXA patients clinically requested to undergo bone mineral density (BMD) analysis. The weight and height of the patients were measured before performing their BMD analysis using the Hologic QDR 4500SL DXA machine. Body mass index (BMI) was calculated as the ratio of the subject's weight to his height squared (kg/m2). The DXA scans were performed in the supine position at the L1 - L4 vertebrea of lumbar spine and the femur hip and completed within approximately ten minutes.

Shortly after DXA scan, multi-frequency complex bioimpedance measurements of the dominant arm were performed at 132 discrete frequencies in the range of 10 kHz to 200 kHz, with Impedimed Multifrequency Analyzer (IMA) (model SFB7) using the four - electrode technique, and repeating each measurement 20 times. The subjects were in sitting position and they were requested to remove all metallic items such as bracelets, necklaces, rings, watches, etc. from the dominant arm [47].

Before the BI measurements, Impedimed Multifrequency Analyzer (IMA) (model SFB7) was calibrated with test cell to ensure standardization of the device (Figure 3.1). The R_{zero} value was recommended to be 604 ± 5 ohms and the R_{inf} value was recommended to be 403 ± 5 ohms [52].

Figure 3.1 Impedimed Multifrequency Analyzer (IMA) (Model SFB7) with Test Cell. [52]

The placement of electrodes was above the 3rd metacarpal bone for the positive current electrode, above the wrist for the positive voltage electrode, on the infraclavicular fossa for the negative voltage electrode, and between shoulder and negative voltage electrode for the negative current electrode (Figure 3.2). A small guarding distance was kept between the electrodes. Following the placement of electrodes, it was checked that the limbs were not adjacent and the patient was not in contact with any metallic surface. The patient remained motionless during the entire measurement process. The bioimpedance spectroscopy (BIS) mode was selected on the IMA device and the patients were alerted before the measurements began. From the Cole-Cole circle of the complex electrical impedance measurements the dominant arm characteristic frequency was calculated.

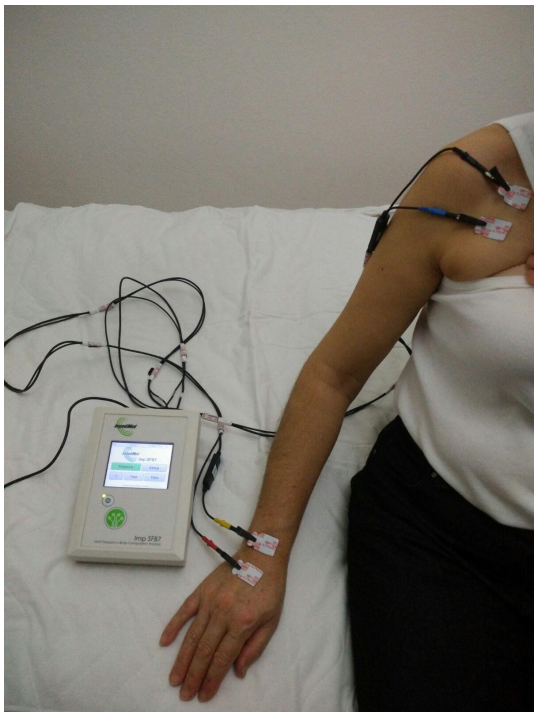

Figure 3.2 Placement of Electrodes and The BIS Device.

T scores, z scores and BMD values were also estimated from DXA results. According to WHO classification system, the participants were classified as normal, osteopenic or osteoporotic based on T and Z scores [3]. Hip and lumbar DXA BMD results were separately analyzed and were classified accordingly as normal, osteopenic or osteoporotic ($T-score \geq -1$ normal, $-1 > T-score > -2.5$ osteopenia, $T-score \leq -2.5$ osteoporosis). In untreated, older postmenopausal women, when the BMD is lower, the fracture risk is greater. In general, fracture risk approximately doubles for each -1 decrease in T-score. A z-score of $<$ -2 represents BMD below the expected age range, while for z $>$ -2 BMD is within the expected age range.

3.2 Statistical Analysis

Statistical analysis was performed using MATLAB R2017b (Mathworks Inc., Natick, MA) program. DXA BMD measurements and characteristic frequencies were compared between all patient subgroups (osteopenic, osteoporotic or normal) defined

based on hip or lumbar BMD values using one-way analysis of variance (ANOVA). If any resultant F-value was statistically significantly high, then Tukey-Kramer test was applied for post-hoc analysis. A total α of 0.05 was contained by using multiple comparison correction. The parameters such as age, age at menopause, weight, height, BMI, bone mineral density, T score, Z score, bone mineral content and characteristic frequency of dominant arm were also compared between the patient subgroups using ANOVA followed by multiple comparison tests. Spearman rank correlation coefficients were computed to assess the correlation between the DXA BMD results and dominant arm characteristic frequencies; and the relationship between hip and lumbar DXA results and BMI, BMD and BMC of the subjects. The Bland Altman method was used to evaluate if there was any systematic bias between the hip and lumbar DXA BMD results. Furthermore, ROC curve analysis was carried out to determine the cut-off values of dominant arm characteristic frequency that was calculated along with the area under curve, sensitivity, specificity and Youden's index.

4. RESULTS

The mean values of total BMD, dominant arm characteristic frequency, BMI, age, and age at menopause of the normal, osteopenic, and osteoporotic patients based on lumbar spine and hip DXA results are displayed in Tables 4.1 and 4.2, respectively.

Table 4.1
The Classification and Statistical Analysis of Patients Based on Lumbar Spine BMD Values.

Parameter	Type	N	Mean +/- SD [Min, Max]	Osteopenia	Osteoporosis
Age (year)	Normal	21	60.2 +/- 9.68 [43, 79]	NS	NS
Age (year)	Osteopenia	22	57.7 +/- 9.1 [45, 86]		NS
Age (year)	Osteoporosis	5	60.4 +/- 8.88 [47, 69]		
Age at Menopause (year)	Normal	21	48.4 +/- 5.2 [40, 58]	NS	NS
Age at Menopause (year)	Osteopenia	22	46.05 +/- 3.6 [40, 52]		NS
Age at Menopause (year)	Osteoporosis	5	45.6 +/- 7.01 [40, 57]		
BMI (kg/m^2)	Normal	21	30.76 +/- 5.51 [19.82, 43.57]	NS	NS
BMI (kg/m^2)	Osteopenia	22	29.97 +/- 6.02 [16.41, 43.42]		NS
BMI (kg/m^2)	Osteoporosis	5	25.25 +/- 1.33 [24.03, 27.33]		
Dominant arm Characteristic Frequency (kHz)	Normal	21	51.72 +/- 8.57 [39.92, 69.57]	NS	$P < 0.005$
Dominant arm Characteristic Frequency (kHz)	Osteopenia	22	55.91 +/- 10.01 [39.96, 75,68]		NS
Dominant arm Characteristic Frequency (kHz)	Osteoporosis	5	65.42 +/- 12.96 [57.98, 88.30]		
Total Lumbar Spine BMD ($g.cm^-2$)	Normal	21	1.048 +/- 0.08 [0.934, 1.259]	$P < 0.001$	$P < 0.001$
Total Lumbar Spine BMD ($g.cm^-2$)	Osteopenia	22	0.855 +/- 0.05 [0.784, 0.929]		$P < 0.001$
Total Lumbar Spine BMD ($g.cm^-2$)	Osteoporosis	5	0.732 +/- 0.04 [0.694, 0.773]		
Total Lumbar Spine Z Scores	Normal	21	1.52 +/- 1.08 [-0.4, 3.6]	$P < 0.001$	$P < 0.001$
Total Lumbar Spine Z Scores	Osteopenia	22	-0.53 +/- 0.6 [-1.5, 0.9]		$P < 0.001$
Total Lumbar Spine Z Scores	Osteoporosis	5	-1.4 +/- 0.89 [-2.7, 0.5]		

Table 4.2
The Classification and Statistical Analysis of Patients Based on Hip BMD Values.

Parameter	Type	N	Mean +/- SD [Min, Max]	Osteopenia	Osteoporosis
Age (year)	Normal	30	60.2 ± 10.1 [45, 86]	NS	NS
Age (year)	Osteopenia	15	58.1 ± 6.2 [49, 69]		NS
Age (year)	Osteoporosis	3	25.8 ± 3.5[43, 68]		
Age at Menopause (year)	Normal	30	47.4 ± 4.9 [40, 58]	NS	NS
Age at Menopause (year)	Osteopenia	15	47.4 ± 4.5 [40, 57]		NS
Age at Menopause (year)	Osteoporosis	3	41.0 ± 1.0[40, 42]		
BMI (kg/m^2)	Normal	30	32.05 ± 4.51 [24.46, 43.57]	$P < 0.001$	NS
BMI (kg/m^2)	Osteopenia	15	26.01 ± 5.81 [16.41, 37.89]		NS
BMI (kg/m^2)	Osteoporosis	3	25.77 ± 3.49 [23.12, 29.72]		
Dominant arm Characteristic Frequency (kHz)	Normal	30	51.63 ± 8.48 [39.92, 71.35]	$P < 0.001$	$P < 0.001$
Dominant arm Characteristic Frequency (kHz)	Osteopenia	15	59.69 ± 11.41 [43.12, 88.30]		NS
Dominant arm Characteristic Frequency (kHz)	Osteoporosis	3	66.40 ± 6.32 [59.14, 70.70]		
Total Hip BMD ($g.cm^-2$)	Normal	30	0.939 ± 0.1 [0.821, 1.232]	$P < 0.001$	$P < 0.001$
Total Hip BMD ($g.cm^-2$)	Osteopenia	15	0.736 ± 0.05 [0.657, 0.805]		$P < 0.001$
Total Hip BMD ($g.cm^-2$)	Osteoporosis	3	0.569 ± 0.07 [0.497, 0.631]		
Total Hip Z Scores	Normal	30	0.9 ± 0.7 [-0.1, 2.6]	$P < 0.001$	$P < 0.001$
Total Hip Z Scores	Osteopenia	15	-2.4 ± 0.86 [-3.30, -1.6]		$P < 0.001$
Total Hip Z Scores	Osteoporosis	3	-2.4 ± 0.86 [-3.30, -1.6]		
Total Hip T Scores	Normal	30	-0.1 ± 0.74 [-1.0, 1.7]	$P < 0.001$	$P < 0.001$
Total Hip T Scores	Osteopenia	15	-1.7 ± 0.39 [-2.3, -1.1]		$P < 0.001$
Total Hip T Scores	Osteoporosis	3	-3.0 ± 0.55 [-3.6, -2.5]		
Total Hip BMC (g)	Normal	30	33.40 ± 7.25 [26, 62.03]	$P < 0.001$	$P < 0.001$
Total Hip BMC (g)	Osteopenia	15	23.86 ± 2.13 [21.4, 28.2]		NS
Total Hip BMC (g)	Osteoporosis	3	20.03 ± 4.04 [15.7, 23.7]		

According to lumbar spine DXA results, 21 participants were classified as normal, 22 as osteopenic and 5 as osteoporotic (Table 4.1). Similarly, according to hip DXA results, 30 participants were classified as normal, 15 as osteopenic and 3 as osteoporotic (Table 4.2). Ages or ages at menopause were not statistically significantly different (NS) between the normal, osteopenic and osteoporotic patients, in either of the lumbar spine or the hip DXA groups. BMI values were higher in normal participants than osteopenic patients ($p < 0.001$) based on hip DXA results. BMD values of both lumbar spine and hip regions decreased with the bone strength ($p < 0.001$). P values at Table 4.1 and Table 4.2 were calculated with Tukey-Kramer post-hoc test. Dominant arm characteristic frequency values of the osteoporotic patients were statistically significantly higher than normal patients for both the lumbar spine ($p < 0.005$)

and hip ($p < 0.001$) subgroups.

The lumbar BMD results were higher than hip BMD results in 32 cases out of 48 participants. 26 patients had the same bone density classification based on both hip and lumbar spine DXA. There was a disagreement between the bone density classifications of hip and lumbar DXA results for the remaining 22 patients (Table 4.3). The hip BMD results were lower than those of lumbar spine region in 5 subjects, and higher in the remaining 17 subjects.

Table 4.3
The Disagreement Between DXA Results from Hip and Lumbar Spine Regions. Lumbar Spine and Hip DXA Results were Consistent in 26 Participants.

Number of Patients	Hip Results	Lumbar Results
3	Osteopenia	Normal
13	Normal	Osteopenia
1	Osteoporosis	Osteopenia
4	Osteopenia	Osteoporosis
1	Osteoporosis	Normal

The Bland Altman test results for the differences of BMD of hip and lumbar body parts of all participants measured by DXA are shown in Figure 4.1. There was not any systematic bias between the two measures according to the mean and standard deviation of the differences ($-0.075 \pm 0.127\ g.cm^{-}2$). However, there were two outliers. The first outlier point had a difference of $0.209 g.cm^{-}2$ between the two measures, and this patient was classified as normal and osteopenic based on hip and lumbar DXA, respectively. BMI of this patient was 31.96 kg/m^2. The second outlier point had a difference of -0.349 $g.cm^{-}2$ between the two measures. This patient was classified as osteoporotic and osteopenic according to the hip and lumbar DXA, respectively. This patient's BMI was 29.72 kg/m^2.

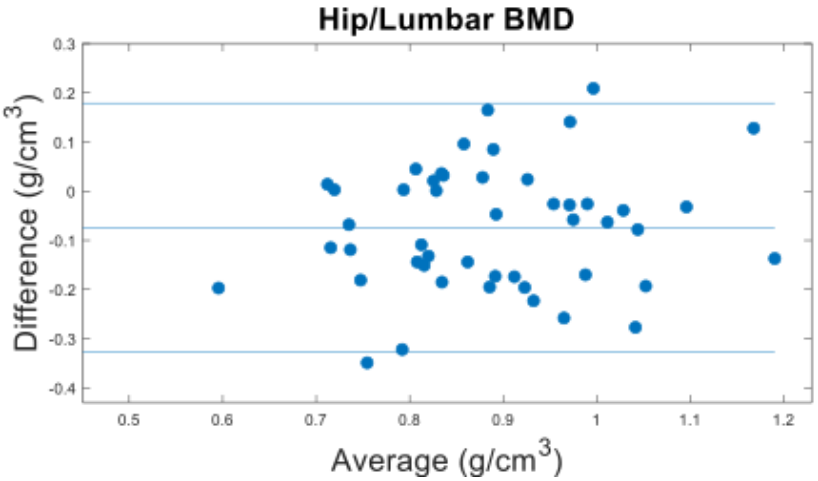

Figure 4.1 Comparison of Hip and Lumbar Spine DXA BMD Results by Bland Altman Method.

According to ranges of BMI (kg/m^2) that are used to describe levels of obesity, participants were classified as 9 normal (BMI $<$ 25.0), 13 as overweight (not obese) (30 $>$ BMI $>$ 25.0); 18 as Class 1 (low - risk) obese (35.0 $>$ BMI $>$ 30.0); 3 as Class 2 (moderate - risk) obese (40.0 $>$ BMI $>$ 35.0) and 3 as Class 3 (high - risk) obese (BMI $>$ 40.0).

The relationships between DXA BMD, dominant arm characteristic frequency, BMI, age and age at menopause are given in Table 4.4. Total hip BMD and total lumbar BMD results were statistically significantly correlated (r = 0.55, p $<$ 0.001). BMD and dominant arm characteristic frequency were inversely correlated. Hip BMD had higher correlation with dominant arm characteristic frequency (r = - 0.53, p $<$ 0.0001), than lumbar BMD (r = - 0.37, p $<$ 0.05). While the correlation between dominant arm characteristic frequency and hip BMD were higher (p = - 0.53, p $<$ 0.001) than hip BMC (r = - 0.43, p $<$ 0.005), the relationship between the dominant arm characteristic frequency and lumbar BMD (r = - 0.37, p $<$ 0.05) were lower than lumbar BMC (r = - 0.47, p $<$ 0.001). The correlation coefficient between hip BMD and dominant arm characteristic frequency (r = -0.36, p = 0.01) were higher than lumbar BMD in the osteopenic groups (r = -0.08 p = 0.73). Although the correlation coefficients between

the BIS results and hip or lumbar BMD results were high in osteoporotic patients, the p values were not statistically significant ($r = 0.5$, $p = 1$ and $r = -0.5$, $p = 0.45$, respectively).

BMC and dominant arm characteristic frequency were inversely associated. Both lumbar BMC ($r = -0.47$, $p < 0.001$) and hip BMC ($r = -0.4340$, $p < 0.005$) were statistically significantly correlated with dominant arm characteristic frequency. BMC and dominant arm characteristic frequency were not statistically significantly correlated in all subgroups due to high p value. No statistically significant correlations were observed between age or age at menopause; BMD or BIS results for all patients or patient subgroups.

Table 4.4
The relationship between lumbar spine or hip BMD, the dominant arm characteristic frequency, age, and age at menopause.

Subjects	Relationship	Number of Subjects	Spearman Correlation Coefficient	p value
All	Total Hip BMD and Total Lumbar BMD	48	0.5503	< 0.0001
All	Total Hip BMD and Characteristic Frequency	48	-0.5311	< 0.0001
All	Total Lumbar BMD and Characteristic Frequency	48	-0.3669	0.0107
All	Total Hip BMC and Characteristic Frequency	48	-0.4340	< 0.005
All	Total Lumbar BMC and Characteristic Frequency	48	-0.4699	< 0.001
Normal (hip)	Total Hip BMD and Characteristic Frequency	30	-0.2598	0.1657
Osteopenia (hip)	Total Hip BMD and Characteristic Frequency	15	-0.3607	0.1870
Osteoporosis (hip)	Total Hip BMD and Characteristic Frequency	3	0.5000	1
Normal (lumbar)	Total Lumbar BMD and Characteristic Frequency	21	-0.2143	0.3493
Osteopenia (lumbar)	Total Lumbar BMD and Characteristic Frequency	22	-0.0785	0.7280
Osteoporosis (lumbar)	Total Lumbar BMD and Characteristic Frequency	5	-0.5000	0.4500
Normal (hip)	Total Hip BMC and Characteristic Frequency	30	-0.0954	0.6146
Osteopenia (hip)	Total Hip BMC and Characteristic Frequency	15	-0.3429	0.2110
Osteoporosis (hip)	Total Hip BMC and Characteristic Frequency	3	1.0000	0.3333
Normal (lumbar)	Total Lumbar BMC and Characteristic Frequency	21	-0.2403	0.2928
Osteopenia (lumbar)	Total Lumbar BMC and Characteristic Frequency	22	-0.4207	0.0524
Osteoporosis (lumbar)	Total Lumbar BMC and Characteristic Frequency	5	-0.6000	0.3500
All	Age and Characteristic Frequency	48	0.1183	0.4233
All	Age and Total Hip BMD	48	-0.1223	0.4076
All	Age and Total Lumbar BMD	48	0.0558	0.7064
All	Age at Menapouse and Characteristic Frequency	48	-0.0250	0.8659
All	Age at Menapouse and Total Hip BMD	48	0.1297	0.3797
All	Age at Menapouse and Total Lumbar BMD	48	0.0752	0.6115
Normal (hip)	Age and Total Hip BMD	30	-0.5150	0.0036
Osteopenia (hip)	Age and Total Hip BMD	15	-0.3396	0.2155
Osteoporosis (hip)	Age and Total Hip BMD	3	-0.5000	1
Normal (lumbar)	Age and Total Lumbar BMD	21	0.0988	0.6700
Osteopenia (lumbar)	Age and Total Lumbar BMD	22	-0.0295	0.8964
Osteoporosis (lumbar)	Age and Total Lumbar BMD	5	0.6669	0.2667
Normal (hip)	Age at Menapouse and Total Hip BMD	30	0.1782	0.3461
Osteopenia (hip)	Age at Menapouse and Total Hip BMD	15	0.3971	0.1427
Osteoporosis (hip)	Age at Menapouse and Total Hip BMD	3	0.5000	1
Normal (lumbar)	Age at Menapouse and Total Lumbar BMD	21	0.1461	0.5275
Osteopenia (lumbar)	Age at Menapouse and Total Lumbar BMD	22	-0.1380	0.5402
Osteoporosis (lumbar)	Age at Menapouse and Total Lumbar BMD	5	-0.4000	0.5167

The results of ROC curve analysis are given in Table 4.5. An optimal cut-off value of 59.14 kHz for dominant arm characteristic frequency resulted in an area under the curve (AUC) of 0.91, with 83.3 percent sensitivity, 100.0 percent specificity and a Youden' s index of 0.8333, to discriminate between normal and osteoporotic patients in hip region ($p < 0.01$). For normal and osteoporotic patients' discrimination in lumbar spine region, an optimal cut-off value of 57.98 kHz was determined, with 0.87 AUC, 76.2 percent sensitivity, 100.0 percent specificity and a Youden' s index of 0.7619 (p

< 0.001). A statistically significant discrimination of normal and osteopenic subjects were observed only in hip region, with an optimal cut-off value of 52.79 kHz with 0.74 AUC, 63.3 percent sensitivity, 80.0 percent specificity and a Youden' s index of 0.4333 ($p < 0.001$). The discrimination of normal against osteopenia and osteoporosis was analyzed and an optimal cut-off value was estimated as 54.0 kHz with 0.76 AUC, 72.2 percent sensitivity, 73.3 percent specificity and a Youden' s index of 0.456 ($p < 0.001$). An optimal cut-off value for the discrimination of osteoporosis against normal and osteopenia based on hip region was found as 58.58 kHz with 0.87 AUC, 100.0 percent sensitivity, 75.6 percent specificity and a Youden's index of 0.756 ($p < 0.005$). ROC analysis of dominant arm characteristic frequencies were statistically significant in all hip classification groups except for the discrimination between osteopenia and osteoporosis.

Table 4.5
ROC Curve Analysis.

	Area Under Curve (AUC) [95 % CI]	Significance level P	Cut-off Dominant Arm Characteristic Frequency (kHz)	Sensitivity (%) [95 % CI]	Specificity (%) [95 % CI]	Youden' s Index
Characteristic Frequency (osteoporosis vs. normal and osteopenia) (hip)	0.87 [0.60-1.00]	< 0.005	58.58	100.0[100.0-100.0]	75.6 [63.0-88.1]	0.756
Characteristic Frequency (osteoporosis vs. normal and osteopenia) (lumbar)	0.80 [0.55-1.0]	0.0085	57.07	100.0 [100.0-100.0]	69.8 [56.0-83.5]	0.698
Characteristic Frequency (normal vs. osteopenia and osteoporosis) (hip)	0.76 [0.62-0.91]	< 0.001	54.0	72.2 [51.5-92.9]	73.3 [57.5-89.2]	0.456
Characteristic Frequency (normal vs. osteopenia and osteoporosis) (lumbar)	0.66 [0.51-0.82]	0.0184	50.43	74.1 [57.5-90.6]	52.4 [31.0-73.7]	0.2654
Characteristic Frequency (normal vs. osteoporosis) (hip)	0.91 [0.79 - 1.0]	< 0.001	59.14	83.3 [70.0 - 96.7]	100.0 [100.0 - 100.0]	0.8333
Characteristic Frequency (normal vs. osteoporosis) (lumbar)	0.87 [0.72 - 1.0]	< 0.001	57.98	76.2 [58.0 - 94.4]	100.0 [100.0 - 100.0]	0.7619
Characteristic Frequency (normal vs. osteopenia) (hip)	0.74 [0.59 - 0.88]	< 0.001	52.79	63.3 [46.1 - 80.6]	80.0 [59.8 - 100.0]	0.4333
Characteristic Frequency (normal vs. osteopenia) (lumbar)	0.62 [0.45 - 0.79]	0.09				
Characteristic Frequency (osteopenia vs. osteoporosis) (hip)	0.78 [0.45 - 1.0]	0.05				
Characteristic Frequency (osteopenia vs. osteoporosis) (lumbar)	0.73 [0.46 - 1.0]	0.05				

5. DISCUSSION

In the assessment of BMD of different parts of the body using DXA, it is possible to obtain different BMD results since the diagnosis is usually based on the area of measurement (hip or lumbar spine), and this might cause confusion [53]. Some radiologists prefer choosing the hip or femoral neck areas as diagnostic reference, while others prefer choosing the area of the lowest BMD [54]. Both hip and lumbar spine areas are effective in estimating hip fractures. Moreover, hip region measurements could previse all fractures. Also, hip region has been reported to be less affected by hormones, medications and degenerative arthritis [54].

It has also been reported 30 percent discrepancies in bone mineral densities between the right and left hips in post-menopausal Women [55, 56]. The relationship between femoral neck and trochanteric areas' BMD measurements is statistically significantly different as shown in a previous study and bilateral hip scan is recommended for a better diagnosis [57].

In our results, it was also observed that 22 out of 48 subjects were classified differently based on hip and lumbar spine DXA measurements, and in 32 participants the lumbar BMD values were higher than hip BMD values. Also, when hip and lumbar spine BMD results of the subjects were compared by the Bland Altman method, it was seen that they were not the same. The subjects, whose lumbar spine and hip BMD results had a disagreement, had high BMI. 17 out of 22 subjects had higher hip BMD than lumbar BMD values. Contrary to the general measurements, the high values of the hip BMD in the mismatches could have been caused by a failure during the lumbar DXA scanning. L4 has the highest BMD in L1 - L4, so the total lumbar BMD results may change due to an incorrect selection of L4 or L1 having too low BMD [58].

Lumbar BMD is highly affected by degenerative arthritis and this might raise

the lumbar spine BMD values. For this reason, while the BMD measurements in other areas decline, the lumbar spine BMD may even rise in patients of 65 years of age and older [54]. It could be concluded that the hip region measurements are more reliable since both hip regions detect all fractures and are less affected of other factors (hormones, medications, degenerative arthritis).

Previous studies have already shown that there exists a correlation between BIA and BMD [13]. BIA and BMD results of both hip and lumbar spine areas were previously found to be correlated in men; meanwhile, hip BMD were found to be associated with bioelectrical impedance results in women [13]. In this study, BIS results were found to be negatively correlated with hip and lumbar BMD. Moreover, with postmenopausal women, a higher correlation coefficient was found between hip BMD results and dominant arm characteristic frequencies compared to lumbar spine BMD results. Also, ROC curve analysis showed that the dominant arm characteristic frequency could be discriminated based on hip subgroups. Only, the discrimination between osteopenia and osteoporotic subgroups did not have a statistically significantly cut-off value among hip subgroups, while among lumbar subgroups, the discrimination ability was significant only between normal and osteoporotic subjects. A previous study showed that the forearm bone mineral content discrimination is same with spinal bone mineral density for vertebral fractures, while the forearm BMC has better discrimination than spinal BMD for peripheral fractures [59].

While measurement protocol was planned, we aimed to optimize the conditions of both the environment and subjects. Measurements was done in the same room and temperature was checked with the help of the air conditioner. It was noted that the participants always stayed in the same position and the device was in a fixed place. However, the measurements could not be achieved at the same time of a day or after a specific activity. These factors could have affected the results. If measurements are made at certain times of a day and in fasting state, like blood measurements, better results could be obtained.

Although the best available evaluation of one's bone density is performed with a DXA scan, the gold-standard method, possible problems may negatively influence DXA results such as different manufacturer's machines or different machines from the same manufacturer or even the same machines from the same manufacturer at different locations, varying machine operators and different anatomical areas in the scan.

The impact of weight and body mass index on bone mineral density is still not fully understood. Some studies have shown that obese or overweight people have lower risk of osteoporosis [60, 61]. Additionally, there exist studies that have shown that weight and high BMI are positively associated with BMD, while other studies have shown that body fat percentage influences BMD negatively [62]. Central or peripheral body fat mass have been reported to be inversely associated with BMD [63]. Visceral fat has also been reported to have negative impact on trabecular bone mass [64]. Here, body mass index was analyzed between groups to better understand the effects of weight and height on the skeletal bones.

Bone loss is accelerated with decreasing estrogen in women after menopause. Also, it is known that bone loss occurs with aging [22]. In this study, age and age at menopause were not correlated with BMD and characteristic frequency. The lack of correlation may have resulted from limited number of subjects. Although a correlation was observed between BIS and DXA measurements, results were not statistically significant to conclude that BIS method could be used in screening for bone mineral deficiency, as a complementary tool to DXA. Due to limited number of subjects, in the statistical results, power was low while p values were high. The characteristic frequencies obtained with the BIS method are influenced by parameters such as fat mass (FM), fat free mass (FFM), intracellular water (ICW) and extracellular water (ECW). BIS studies should be carried out with more subjects by including more parameters.

6. CONCLUSION

This thesis was conducted to understand the relationship between characteristic frequency gathered from BIA and bone level. To understand this relation, a comparison was made between DXA and BIA results. It was observed that there is a negative moderate correlation between these two methods. One of the main objective of this research was to define a new methodology for osteoporosis diagnosis.

A statistically significant negative correlation was observed between the characteristic frequency of the dominant arm and both hip and lumbar spine DXA BMD, with a higher correlation with the hip. Being simple, cheap, safe and easy to use, bioelectrical impedance spectroscopy could be suggested as a screening alternative tool in assessing the bone mineral deficiency in post-menopausal women.

6.1 Future Work

Future plans about this study might be enhancing the BIA measurement in a field of analyzing bone level. To enhance these measurements, BIA should perform in a more standardized way. This standardization could be achieved by taking measurements after the same activity, same time interval from meal and physical activity. For example, measurements could be taken when subjects are hungry in the early morning and after they stand still for fifteen minute before the measurement. This waiting period might enable subjects to be at the same physical condition.

Each BIA device has different equations to estimate body compositions. So, setting same specific equations for bone mineral level assessment could enhance evaluation of bone status. Also, biologic inheritance influences bone mineral density and quality. When these equations are formed according to this inheritance, the more reliable estimations could be made. One of our future plans is first investigating Turkish

population's inheritance and setting a procedure according to this population.

Standardization is an important factor for all diagnostic tools. To achieve a standardized BIA, only one subject could be repeatedly scanned within a day and between days for a week. Within day measurements could be done at the beginning of each hour, while between day measurements could be done at the same time everyday such as, at 10 a.m. These measurements should also be repeated with different devices to compare the performance of the devices.

It is known that bone mineral density and quality measurements are affected by fat or fat free mass. The measurements could be improved by adding body composition information. Combining body composition and characteristic frequency measurements could help with accomplishing a better bone level estimation. The devices of BIA measure body compositions from whole body measurements. Both characteristic frequency and body compositions could be gathered in a single examination and they could be used in a single equation for a better understanding of bone quality.

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