Occurrence of Osteoporosis Among Menopausal Women in Gaza Strip

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Submitted in Partial Fulfillment for the Master Degree of Science in Biological Sciences-Medical Technology

2008
Declaration

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Dedication

To the person who taught me patience, strife, and pushed me towards success in life and give me all care happiness.

To my father Mahmoud Hania,

Mother Amal Hania

Sisters

Hadeel

Hanaa

Heba
Acknowledgements

First of all, I thank God for the beneficent and most merciful.

I wish to thank my supervisors Prof Mohammad E. Shubair for his support, assistance, and guidance. In addition, special thanks are due to the teaching staff of Biological Sciences Master Program and to all of my colleagues in Medical Technology and Biology departments- Islamic University of Gaza.

I would like to express my sincere appreciation to the Palestinian Ministry of Health represented by all the staff in the European hospital, Al-shifa hospital (physicians and staff nurses team) and special thanks for the staff of laboratory in the European hospital.

Thanks to the Balsam laboratory staff for their effort, kindness, and support in lab work.

I would like also express my thanks to all friends in the master program for the great and lovely time that we spent together.

I owe my deepest gratitude to my father, mother, and my sisters for their love and caring throughout my life.
Abstract

Occurrence of Osteoporosis Among Menopausal Women in Gaza Strip

Osteoporosis is a systemic disease of the skeleton, characterized by low bone mass and alterations in the micro-architecture of the bone tissue that lead to an increase in brittleness with the ensuing predisposition to bone fractures. Classified into primary and secondary osteoporosis. Global statistics shows that women are more exposed to this disease than men and in particular at menopause.

Extensive research has been carried out in developed concerning osteoporosis; unfortunately little information are available in our area. The current research aims at studying risk factors which may lead to this disease, in addition, we intend to evaluate the efficacy of various laboratory investigations participating in prefect diagnosis and follow up of this disease, these are; serum calcium, magnesium, sodium, alkaline phosphatase, estradiol and newly introduced test in our area, osteocalcin.

A group of number 96 women who attend orthopedic clinics suffering from osteoporosis were chosen, their age range was 40-60 years, another controls of number group "osteoporosis free" was chosen of comparable age. The total number of each group was 96 women.

Results showed statistically significant differences between the levels of sodium, magnesium and alkaline phosphatase but the levels of calcium and phosphorous were not significant in comparison to the control group levels.

Results of estradiol and osteocalcin were statistically significant "p<0.05" which emphasize the importance of conducting these two test for such cases.
The questionnaire results showed unawareness of women concerning osteoporosis as their daily practices proved; they do not drink milk regularly, they do not practice sports, exposure to sunlight is minimal, drinking increased amounts of coffee and soft drinks, in addition, they were not aware of hereditary determination which may lead to this disease.

we recommend to introduce both estradiol and osteocalcin assays in the laboratories of MOH as they have proved to be valuable. Lastly we recommend to establish scientific society aiming at spreading the relevant information about this disease and support research.

Key words: osteoporosis, menopause, osteocalcin, estradiol, Gaza Strip.
الخلاصة

" هشاشة العظام لدى النساء بعد سن اليأس في قطاع غزة "

يعتبر مرض هشاشة العظام " ترقق العظام " من أمراض تحيط الهيكل العظمي وتسبب خلايا في النسيج العظام مما يؤدي لزيادة الترقق وحروق الكسور. يصنف هذا المرض إلى مرحلتين، هما البداية والثانوية وتتطلب الإحصائيات أن معدل إصابة النساء بهذا المرض أكثر من الرجال خاصة بعد سن اليأس . و لقد أجريت العديد من الأبحاث على هذا المرض في كثير من الدول لا حجم المعلومات المتوفرة عن هذا المرض في منطقتنا يعتبر ضئيلاً با لمقارنة بالمعلومات في العديد من الدول.

لذا فإن هذا البحث يهدف إلى دراسة العوامل التي تساهم في تسبب هذا المرض. كما أن البحث يهدف إلى تقييم فعالية الفحوصات المخبرية التي تساعد في تشخيص وسير المرض " عناصر الكالسيوم، الماغنسيوم، الصوديوم، أيزوم الفوسفات القلوي، هرمون الاستروديال " وكذلك الاستيوكالسيس الذي يعمل لأول مرة في قطاع غزة.

تم اختيار مجموعة من النساء الملتزمات على عادات العظام و الاسم يعانيمن من مرض هشاشة العظام تتراوح أعمارهن بين 30-60 كما تم اختيار العينة الضابطة لنفس الفئة العمرية من النساء بشرط خلوها من أي مرض في العظام. وكان عدد السيدات اللواتي يعانين من هشاشة العظام 92 عينة وعدد السيدات اللواتي لا يعانين من هشاشة العظام 92 عينة.

ولدى مقارنة المرضى بالعينة الضابطة، تبين وجود دلالة إحصائية لكل من الصوديوم ، الماغنسيوم ، أيزوم الفوسفات القلوي بينما لم تكن هناك دلالة إحصائية لمستويي الكالسيوم والفسفور. كما أظهرت النتائج وجود دلالة إحصائية لهرمون الاستروديال و الاختبار الجديد " لاستيوكالسيس " مما يعزز أهمية إجراء هذه الفحوص للكثير من هذه الحالات.

وقد ظهر من خلال الاستبانة قلة الوعي لدى النساء المرضى بهذا المرض مثلا في شرب اللبن، وعدم ممارسة الرياضة وعدم التعرض للشمس وتناول المشروبات الغازية والمكحول بإفراط. كما لم يكن لدىهن وعي كافي بالعوامل الوراثية التي تسبب في هذا المرض . و من خلال استعراض النتائج التي خلص إليها هذا البحث فإننا نوصي ب.mybatisplus توعية صحية للنساء المعرضات لإصابات هشاشة العظام والتأكيد على أهمية الفحوصات الإكلينيكية المخبرية والأشعة والتي يجب أن تبدأ في سن مبكرة حتى يمكن المحافظة على سلامة العظام كما نوصى بالتعرض لأشعة الشمس وتناولكم كميات كافية من الكالسيوم وفيتامين د والعناية بذكارنها مع الأدوية خاصة مجموعة الكورتيزون ونوصي أيضا بإنشاء جمعية عملية هدفها التعريف بخطورة هذا المرض وزيادة الوعي لدى النساء ودعم الأبحاث الخاصة بها كما هو معمول بذلك في الدول المتقدمة. وأخيرا فإننا نوصي فحص الاستروديال والاستيوكالسيس في مختبرات وزارة الصحة لما لها من أهمية تشخيصه خاصة.

VI
# Table of contents

<table>
<thead>
<tr>
<th>Items</th>
<th>pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Declaration</td>
<td>I</td>
</tr>
<tr>
<td>Dedication</td>
<td>II</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>III</td>
</tr>
<tr>
<td>Abstract (English)</td>
<td>IV</td>
</tr>
<tr>
<td>Abstract (Arabic)</td>
<td>VI</td>
</tr>
<tr>
<td>List of contents</td>
<td>XIII</td>
</tr>
<tr>
<td>List of tables</td>
<td>XIV- XV</td>
</tr>
<tr>
<td>List of figures</td>
<td>XVI</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>XVI- XVIII</td>
</tr>
</tbody>
</table>

**Chapter One : Introduction**

1.1 Overview ................................................................. 1- 3
1.2 Statement of the osteoporosis .................................  3
1.3 Aims of study .......................................................... 4
1.4 The significance ....................................................... 4

**Chapter Two : Review of the literature** ...........................

2.1 Bone definition ............................................................. 5
2.2 Bone remodeling .......................................................... 5
2.3 Peak bone mass .......................................................... 5
2.4 Loss of bone density ..................................................... 6
2.5. Definitions: Osteoporosis ................................................................. 6

2.6. Role of Hormones in bone formation.................................................... 7

2.6.1. Parathyroid hormone ...................................................................... 7

2.6.2. Activated vitamin D ...................................................................... 7

2.6.3. Sex hormones .............................................................................. 7

2.6.3.1. Estrogen ................................................................................. 8

2.6.3.2. Androgens ............................................................................. 8

2.7. Etiology of osteoporosis ................................................................... 8-9

2.8. Osteoporosis Symptoms .................................................................. 9-10

2.9. Classification of systemic osteoporosis .............................................. 10

2.9.1. Primary osteoporosis ................................................................. 10

2.9.1.1. Classification of primary osteoporosis ....................................... 11

2.9.2. Secondary osteoporosis ............................................................... 11

2.9.3. Idiopathic juvenile osteoporosis ................................................... 11

2.10. Risk factors of osteoporosis .............................................................. 12

2.10.1. Risk factors of osteoporosis that cannot be changed or influenced................................................................. 12

2.10.1.1 Being female ............................................................................ 12

2.10.1.2. Advancing age ...................................................................... 12

2.10.1.3. Race/ethnicity estimates ......................................................... 13

2.10.1.4. Menopause ............................................................................ 14
2.10.1.5. Family history of osteoporosis......................................... 14
2.10.1.6. Amenorrhea ................................................................. 14
2.10.1.7. Nulliparity, pregnancy, lactation, and osteoporosis........... 14-15
2.10.2. Risk factors of osteoporosis that can be changed or influenced............................................................... 15
   2.10.2.1. Diet .......................................................... 15
      A. protein......................................................................................... 15
      B. Sugar ......................................................................................... 16
      C. Alcohol ...................................................................................... 16
      D. Phosphorus ................................................................................ 16
      E. Cola drinks ................................................................................ 16
      F. Caffeine ....................................................................................... 17
      G. Sodium ...................................................................................... 17
      H. Low dietary calcium intake .................................................... 17-18
   2.10.2.2. Smoking ........................................................................... 18
   2.10.2.3. Lack of exposure to sunlight ........................................... 18
   2.10.2.4. Lack of exercise ............................................................... 18
   2.10.2.5. Malnutrition ................................................................. 19
   2.10.2.6. Depression ............................................................... 19
   2.10.2.7. Excess vitamin A ........................................................... 19
   2.10.2.8. The effect of corticoids on bone ...................................... 19-20
2.11. Genetic and osteoporosis......................................................... 20-21
2.12. Epidemiology of osteoporosis................................................... 21-23
2.13. Menopause ............................................................................. 23
   2.13.1. Definition of menopause.................................................. 23
   2.13.2. Stages of menopause......................................................... 24
      2.13.2.1. Perimenopause ......................................................... 24
2.13.2.2. Menopause ................................................................. 24
2.13.2.3. Postmenopause ......................................................... 24
2.13.3. Types of menopause ...................................................... 24
  2.13.3.1. Premature menopause ............................................ 24-25
  2.13.3.2. Surgical menopause ................................................. 25
  2.13.3.3. Natural menopause .................................................. 25
2.13.4. Symptoms of menopause .............................................. 25
2.13.5. Causes of menopause .................................................. 26
2.13.6. Level of estrogen in women life ..................................... 26
2.13.7. Global statistics of women at menopause ......................... 27
2.14. Diagnosis of osteoporosis in menopause women ................... 27
  2.14.1. History and physical examination ............................... 27
  2.14.2. Bone mineral density ................................................ 28
  2.14.3. Common bone mineral density tests ............................ 28
  2.14.4. Various BMD tests .................................................... 29
  2.14.5. Interpretation of BMD results ..................................... 29
  2.14.6. Clinical laboratory investigations ............................... 30
  2.14.7. The most common laboratory tests .............................. 31
  2.14.8. Bone markers .......................................................... 31
    2.14.8.1. Osteocalcin ....................................................... 31-32
    2.14.8.2. Alkaline phosphatase ........................................ 32
    2.14.8.3. Magnesium ...................................................... 32
    2.14.8.4. Blood calcium and phosphorus ............................. 33
    2.14.8.5. Estradiol levels ............................................... 33
2.15. Basic prevention ........................................................ 33
  2.15.1. Diet ..................................................................... 33
    2.15.1.1. Calcium intake ................................................. 33
    2.15.1.2. Vitamin D ......................................................... 33-34
2.15.1.3. Soybeans in prevention of osteoporosis ............................................ 34
2.15.1.4. Onion in prevention of osteoporosis .............................................. 34
2.15.1.5. Green tea ......................................................................................... 35
2.15.1.6. Limit caffeine, protein, vitamin A and sodium ................................ 35
2.15.2. Exercise .............................................................................................. 35
2.15.3. Prevention falls .................................................................................. 36
2.16. Drugs for the treatment of osteoporosis ............................................... 36
2.17. Previous study ....................................................................................... 36-37

Chapter three : Materials and Methods ......................................................

3.1. Study design ............................................................................................ 38
3.2. Study population ..................................................................................... 38
3.2.1. Patients group .................................................................................... 38
3.2.2. Controls group .................................................................................. 38
3.3. Selection of subjects ............................................................................ 38
3.4. Questionnaire interview ......................................................................... 38
3.5. Inclusion criteria .................................................................................... 38-39
3.6. Subjects identification ........................................................................... 39
3.7. Ethical considerations ........................................................................... 39
3.8. Materials and reagents .......................................................................... 39- 40
3.9. Instruments ............................................................................................. 40
3.10. Blood sampling and processing .......................................................... 40
  3.10.1. Osteocalcin ......................................................................................... 40- 42
  3.10.2. Calcium ........................................................................................... 43-44
  3.10.3. Phosphate .......................................................................................... 44-45
  3.10.4. Alkaline phosphatase ...................................................................... 45-47
  3.10.5. Magnesium ....................................................................................... 47-48
  3.10.6. Sodium ............................................................................................ 48-49
  3.10.7. Estradiol .......................................................................................... 49-50
3.11. Statistical analysis ........................................................................................................51

Chapter four : Results ........................................................................................................

4.1. Demographic characteristics of the study population (patients n=96 and controls n=96).................................................................................................................. 52

4.2. Risk factors causes osteoporosis among menopausal women........... 52-61

4.3. Results of sign test for magnesium, estradiol and osteocalcin.............62-63

4.4. T-test for Ca/P ratio.......................................................................................................63

4.5. Mann-Whitney U test for magnesium, estradiol and osteocalcin .........64

4.6. T-Test is used to examine the mean differences of the Calcium / Phosphorus ratio between patients and controls group..............................................................65

4.7. Results of person correlation coefficient.................................................................66

Chapter five : Discussion .................................................................................................

5.1. Overview ......................................................................................................................67

5.2. Serum calcium ..........................................................................................................68

5.3. Serum phosphorus ....................................................................................................68

5.4. Serum Sodium .........................................................................................................68-69

5.5. Serum Magnesium ....................................................................................................69

5.6. Serum alkaline phosphatase ..................................................................................70

5.7. Serum estradiol ........................................................................................................70-71

5.8. Serum osteocalcin .....................................................................................................71

5.9. Serum ratio Ca/P .......................................................................................................71-72

5.10. Risk factors from questionnaire interview for patient group ...............72
Chapter six : Conclusions and Recommendations

6.1. Conclusions........................................................................................................73

6.2. Recommendations............................................................................................74

References.............................................................................................................75-88

Appendix : .............................................................................................................

Appendix A: Questionnaire Arabic .......................................................................89-90

Appendix B: Questionnaire English ......................................................................90-93

Appendix C: European Hospital Approval form ..................................................94

Appendix D : Al_ Shifa Hospital Approval form ....................................................95
<table>
<thead>
<tr>
<th>Table No.</th>
<th>Description</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 2.1</td>
<td>Comparison of type I and type II osteoporosis</td>
<td>11</td>
</tr>
<tr>
<td>Table 2.2</td>
<td>Estimated Percent of Adults Aged 50 Years and Over to Have Osteoporosis or Low Bone Mass by Ethnic Group, In USA</td>
<td>13</td>
</tr>
<tr>
<td>Table 2.3</td>
<td>Prevalence rate for osteoporosis</td>
<td>22-23</td>
</tr>
<tr>
<td>Table 2.4</td>
<td>Tests to Evaluate Bone Health</td>
<td>29</td>
</tr>
<tr>
<td>Table 2.5</td>
<td>Comparison of Different Modalities for Assessing Bone Fracture Risk</td>
<td>29</td>
</tr>
<tr>
<td>Table 2.6</td>
<td>T-scores</td>
<td>30</td>
</tr>
<tr>
<td>Table 4.1</td>
<td>Demographic characteristics of the study population (patients n=96 and controls n=96)</td>
<td>52</td>
</tr>
<tr>
<td>Table 4.2.1</td>
<td>Distribution of Osteoporotic patients (n=84) according to LMP</td>
<td>52</td>
</tr>
<tr>
<td>Table 4.2.2</td>
<td>Activities causing joint pain among osteoporotic women n=84</td>
<td>53</td>
</tr>
<tr>
<td>Table 4.2.3</td>
<td>Average time of practicing physical activities/min/day by osteoporotic patients n=85</td>
<td>53</td>
</tr>
<tr>
<td>Table 4.2.4</td>
<td>Load of daily work of osteoporotic women (n=85)</td>
<td>54</td>
</tr>
<tr>
<td>Table 4.2.5</td>
<td>The reason to stop working or walking among osteoporotic women (n=85)</td>
<td>54</td>
</tr>
<tr>
<td>Table 4.2.6</td>
<td>Different risk factors leading to osteoporosis in menopausal women (n=96)</td>
<td>55-56</td>
</tr>
<tr>
<td>Table 4.2.7</td>
<td>Different risk factors leading to osteoporosis in menopausal women with no recorded osteoporosis (n=96)</td>
<td>57-58</td>
</tr>
<tr>
<td>Table 4.2.8</td>
<td>Difference between proportions of healthy and patients groups</td>
<td>59-60</td>
</tr>
<tr>
<td>Table 4.3</td>
<td>Arithmetic mean of variables for patient n=96 and control groups n=96</td>
<td>60</td>
</tr>
<tr>
<td>Table 4.4</td>
<td>Arithmetic mean of estradiol and osteocalcin for patient n=96 and control n=96</td>
<td>61</td>
</tr>
<tr>
<td>Table 4.5</td>
<td>Arithmetic mean of magnesium, estradiol, osteocalcin Ca/P and alkaline phosphatase for patient and control groups (Kolmogorov-Smirnov Test)</td>
<td>61</td>
</tr>
</tbody>
</table>
Table 4.6. Sign test for magnesium ................................................................. 62
Table 4.7. Sign test for estradiol ................................................................. 62
Table 4.8. Sign test for osteocalcin .............................................................. 63
Table 4.9. T-test for Calcium / Phosphorus ratio ...................................... 63
Table 4.10. Mean rank by Mann-Whitney test .......................................... 64
Table 4.11. Comparison of means for magnesium, estradiol, and osteocalcin between patient and control groups ......................................................... 64
Table 4.12. Comparison of means for Calcium / Phosphorus ratio between patient and control groups ................................................................. 65
Table 4.13. Relationships between osteocalcin and other variables ............. 66
List of figures

**Figure 2.1.** Changes of bone mass in women and men in relation to age.............6

**Figure 2.2.** Normal Bone (a) vs. (b) Osteoporotic Bone.................................6

**Figure 2.3.** Estrogen levels during women life..............................................27
List of abbreviations

BMD : Bone mineral density.
Ca : Calcium.
DEXA : Dual-energy x-ray Absorptiometry.
EGCG : Epigallocatechin gallate.
FSH : Follicle Stimulating Hormone.
FDA : Food and Drug Administration.
GMCSF : Granulocyte/ Macrophage Colony Stimulating Factor.
GEDTA : Glycoletherdiamine- tetraacetic acid.
HRT : Hormone replacement therapy.
IU/L : International unit per liter
Mg : Magnesium.
mg/dl : Milligrams per deciliter
mEq/L : Milliequivalents per liter
NIH : National Institutes of Health.
Na : Sodium.
PBM : Peak Bone Mass.
PTH : Parathyroid Hormone.
pQCT : Peripheral Quantitative Computed Tomography.
Pg/dl : Pico gram per deciliter
QUS : Quantitative ultrasound.
QCT : Quantitative Computed Tomography.
RA : Radiographic Absorptiometry.
SERMs: Selective Estrogen Receptor Modulators.

SD: Standard deviation.

T score: Skeletal mass given as SD units away from the mean value for a young healthy population.

TNF-a: Tumor Necrosis Factor.

TMB: Tetramethylbenzidine.

TGF-B: Transforming growth factor B.

UVB: Ultraviolet-B.

VDR: Vitamin D Receptor.

WHO: World Health Organization

Z score: Skeletal mass given as SD units away from the mean value for an age-matched cohort.
Chapter One

Introduction
Introduction

1.1. Overview

Osteoporosis is a systemic disease of the skeleton, characterized by low bone mass and alterations in the micro-architecture of the bone tissue that lead to an increase in brittleness with the ensuing predisposition to bone fractures (1). Osteoporosis is a “silent killer” that millions of people around the world suffer from (2), and it is important due to its morbidity, mortality, adverse effects on the quality of life and the extra costs imposed on the patient and the society (3). The increase of life expectancy and so the old age in the society in developing countries such as the Middle East has led to an increase in the prevalence of osteoporosis and its following fractures in the area (4).

Osteoporosis is classified into primary and secondary ones. Primary osteoporosis is manifested by deterioration of bone mass that is unassociated with other chronic illness and is related to aging and decreased gonadal function. Therefore, early menopause or perimenopause estrogen deficiency states may hasten the development of primary osteoporosis. Prolonged periods of inadequate calcium intake, sedentary lifestyle and tobacco and alcohol abuse also contribute to this condition. Secondary osteoporosis results from chronic conditions that contribute significantly to accelerated bone loss. These chronic conditions include endogenous and exogenous thyroxine excess, hyperparathyroidism, malignancies, gastrointestinal diseases, medications, renal failure and connective tissue diseases (5).

Many people with osteoporosis have several risk factors, but others who develop the disease have no known risk factors. There are some factors which cannot be changed while others could be changed. Risk factors which cannot be changed include gender, age, body size, ethnicity. Family history and other factors that can be changed include sex hormones, anorexia nervosa, calcium and vitamin D intake, medication use, lifestyle, cigarette smoking, and alcohol intake (6).

The first sign of osteoporosis is often a bone fracture. This is virtually always the case with osteoporosis of the hip. Sometimes the fracture happens due to a relatively minor fall (7). Back pain can be a symptom of a wide variety of
problems, including osteoporosis of the spine. With loss of bone mineral density, the vertebral bones in the spine can gradually decrease in height and become deformed, but the most serious concern is a compression fracture in which a vertebral body collapses. This can affect nerves and lead to pain and serious disability. Less commonly, decreased height of the main vertebral bodies can cause the bony protrusions (spinal processes) of the vertebrae to make painful contact with each other (8).

The diagnosis of osteoporosis is usually made by a qualified physician using a combination of medical history, physical evaluation, laboratory tests and bone mass measurement testing. Tests include; Bone Mineral Density Testing, Dual Energy X-ray Absorptiometry (DEXA), Peripheral Dual Energy X-ray Absorptiometry (pDXA), Single Energy X-ray Absorptiometry (SX), Peripheral Quantitative Computed Tomography (pQCT), Radiographic Absorptiometry (RA), Quantitative Computed Tomography (QCT) and Quantitative Ultrasound (QUS) (9).

Osteoporotic fractures are major cause of illness and death in older women, particularly menopausal ones (10).

Natural menopause is quite specifically defined and is confirmed when a woman has not had a menstrual period for a 12-month period. Women go through different phases of menopause, including Perimenopause, menopausal, and postmenopausal periods. When a woman approaches menopause, she may begin to experience a variety of symptoms. While 75% of women will experience hot flashes, not all women will experience all the symptoms of menopause, nor will they be felt to the same degree. Some women will have virtually no symptoms at all, while others will feel a significant impact on their quality of life. These symptoms are hot flashes, mood, memory, and sleep disturbances (11,12).

Each year about 500,000 American women suffer from fracture of vertebrae and about 300,000 of hip fracture. Nationwide, treatment for osteoporotic fractures costs up to $10 billion per year, with hip fractures being the most expensive. Between 12 and 20% of those who suffer a hip fracture do not
survive the 6 months after the fracture. At least half of those who do survive require help in performing daily living activities, and 15 to 25% will need to enter a long-term care facility. Older patients are rarely given the chance for full rehabilitation after a fall. However, with adequate time and care provided in rehabilitation, many people can regain their independence and return to their previous activities (13). The number of recorded osteoporosis cases in the Gaza Strip was 136,396 in 2004 (14,15).

The prevention of osteoporosis is a lifetime process. Most bone mass is developed before the age of 30. Thereafter, the challenge is to retain the bone mass one has. Efforts to assure the development of adequate bone mass throughout the lifespan should begin with children and adolescents through the consumption of calcium-rich and vitamin D-rich diets and through frequent weight-bearing exercise. In mid-life, continued consumption of calcium and vitamin D and physical activity are important. A healthy lifestyle without smoking or excessive alcohol is helpful. If necessary, calcium supplements should be considered (5). Pharmacological interventions with drug administration include the oral bisphosphonates, calcitonin, parathyroid hormone and selective estrogen receptor modulators. Food drug administration has withdrawn approval of estrogen or hormone therapy for treatment of osteoporosis but has continued approval of their use for osteoporosis prevention in selected postmenopausal women (16).

1.2. Statement of the problem:

Osteoporosis has been extensively investigated in the developed countries but less knowledge is available about the magnitude of this problems in developing countries. Although osteoporosis is recorded among menopausal women in Gaza Strip, no published data are available on the disease.
1.3. Aims of the study

The overall aim of the study is to assess osteoporosis among menopausal women in Gaza Strip. The specific objectives are:

1. To study the magnitude of osteoporosis conditions at menopause in Gaza Strip.

2. To evaluate different laboratory tests used for the diagnosis of osteoporosis

3. To draw the attention of women at menopause of the consequences of osteoporosis and provide them with instructions to prevent the onset of osteoporosis.

1.4. The significance

This study the first one in our area according to the knowledge of the researcher. It aims at increasing women awareness of the consequence of osteoporosis. The research focuses on the application of these data to stratifying women by risk factors. Introduction of new laboratory investigations like osteocalcin to help physicians in diagnosis and influence treatment decisions, and ultimately reduce fracture outcome. This study will bring this serious medical problem into focus, so policy makers in health sector may consider launching programs for dealing with it.
Chapter Two

Review of literature
Review of the literature

2.1. Bone definition

Bone is a living tissue that supports our muscles, protects vital internal organs, and stores most of the body’s calcium. It consists mainly of a framework of tough, elastic fibers of a protein called collagen and crystals of calcium phosphate mineral that harden and strengthen the framework. The combination of collagen and calcium phosphate makes bones strong yet flexible to hold up under stress. Bone also contains living cells, named osteoblasts and osteoclasts (17).

2.2. Bone remodeling

Bone remodeling is the process of building the skeleton and continuously reshaping it to respond to internal and external signals. It is carried out by osteoblasts that form bone and osteoclasts that break down bone. In remodeling there is an important local interaction between osteoblasts or their precursors (the cells that will develop into osteoblasts by acquiring more specialized functions, a process called differentiation) and osteoclasts or their precursors. Since remodeling is the main way that bone changes in adults and abnormalities in remodeling are the primary cause of bone disease, it is critically important to understand this process (18).

2.3. Peak bone mass

Peak bone mass (PBM) is defined as the amount of bony tissue present at the end of skeletal maturation (19). Bone strength is mainly determined by volumetric density, i.e., the amount of bony tissue per unit of volume, by outer bone dimensions (20). It is generally accepted that fractures result from low bone mass. Bone mass accounts for 75-85% of the variance in the ultimate strength of bone tissue, and such measurements also provide an accurate indication of whole bone strength (21). Figure 2.1 shows changes of bone mass in both sexes in relation to age.
2.4. Loss of bone density

In women, and as shown in figure 2.1, there tends to be minimal change in total bone mass between age 30 and menopause. But in the first few years after menopause, most women experience rapid bone loss, a "withdrawal" from the bone bank account, which then slows but continues throughout the postmenopausal years. This loss of bone mass can lead to osteoporosis (23).

2.5. Osteoporosis can be defined as:

1- A systemic skeletal disease characterized by low bone mass and micro-architectural deterioration of bone tissue with a resultant increase in fragility and risk of fracture (24), or
2- A skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture. Bone strength reflects the integration of two main features; bone density and bone quality (25).

Figure 2.2 Normal Bone (a) vs. (b) Osteoporotic Bone (30)
2.6. Role of Hormones in bone formation

2.6.1. Parathyroid hormone

Parathyroid hormone (PTH) plays an important role in extra-cellular calcium homeostasis and acts on the skeleton to stimulate bone turnover. PTH is a stimulator of bone resorption to make calcium available to maintain extra-cellular calcium levels. It is thought to act on osteoclasts to stimulate release of a mediator which then promotes bone resorption. PTH is also known to have a catabolic effect on bone, possibly mediated through other growth factors stimulating osteoblasts proliferation. Alterations in PTH concentration will therefore have effects on bone homeostasis and remodeling. PTH levels have been found to increase gradually with age, and may lead to an increase in bone turnover and consequently to the increased bone loss seen with age. PTH levels will rise in response to low plasma calcium and the high level found in hyperparathyroidism is an important cause of secondary osteoporosis (26).

2.6.2. Activated vitamin D

Activated vitamin D (1,25 dihydroxycholecalciferol) also has an active role in regulating extra-cellular calcium homeostasis, increasing intestinal calcium absorption, minimizing loss of calcium by the kidney, and stimulating bone resorption when necessary. and calcium resorption from the kidney. It is required for normal bone turnover and vitamin D deficiency has been found to correlate with a low BMD (27).

2.6.3. Sex hormones

Sex hormones are important regulators of bone remodeling and in post-menopausal females estrogen deficiency makes the major contribution to the cause of osteoporosis.
2.6.3.1. Estrogen:  
Estrogen receptors have been demonstrated on cell lines of both osteoblasts and osteoclasts. However, estrogen does not appear to act directly at these sites but appears to be mediated through locally produced cytokines, mainly through changes in interleukin-1, interleukin-6, tumor necrosis factor (TNF-α) and granulocyte/macrophage colony stimulating factor (GMCSF). It appears that estrogen deficiency allows greater expression of these cytokines, all of which are associated with increased stimulation of bone resorption which then leads to increased bone loss and a reduction in BMD (28).

2.6.3.2. Androgens:  
Androgens, can directly affect and modulate bone cell function. Androgen receptors are found on osteoblasts cell lines and they can cause osteoblasts proliferation. Hypogonadal men, in common with post-menopausal women, have decreased calcium absorption and low vitamin D levels. The replacement of androgens with testosterone can correct these abnormalities, suggesting again that sex hormones are required for the maintenance of bone health (29). Figure 2.2 shows the difference between the healthy bone and the osteoporotic bone.

2.7. Etiology of osteoporosis  
The final clinical outcome of the osteoporotic process is a fracture, which can occur as a result of minimal trauma or even spontaneously. At present low bone mass is regarded as the main contributor to bone fragility, but possible qualitative changes in the bone matrix must also be considered. Two factors which determine the level of bone mass at any age are the obtained peak bone mass and duration and rate of bone loss. Peak bone mass is achieved during the first three decades of life. Genetic and nutritional factors as well as mechanical stress on the skeleton obviously play crucial roles in determining peak bone mass. Two phases of bone loss age-related and menopause related dictate the final bone mass at old age. Postmenopausal osteoporosis is a particular example of unbalanced bone resorption leading to net bone loss. An increasing number of systemic and
local factors have been found to participate in the regulation of bone remodeling (31).

2.8. Osteoporosis Symptoms

The osteoporosis condition can operate silently for decades, because osteoporosis doesn’t cause symptoms unless bone fractures. Some osteoporosis fractures may escape detection until years later. Therefore, patients may not be aware of their osteoporosis until they suffer a painful fracture. Then the symptoms are related to the location of the fractures(32).

**Early symptoms:** In many cases there is no indication of gradual bone loss. Many people have no early symptoms until they fracture a bone.

**Muscular aches and bone tenderness:** As the disease progresses to the later stages of osteoporosis symptoms may include:

- Neck pain
- Muscle pain
- Bone tenderness

**Fractures:** The most common symptom of osteoporosis is a broken “fracture” bone. Osteoporosis thins and weakens the bones, increasing the risk that a minor injury will result in a bone fracture. Individuals with osteoporosis can suffer from a fracture from something as simple as a cough, sneeze or bumping into a chair.

**Spinal deformities:** Spinal deformities such as a stooped posture or Kyphosis can be a symptom of osteoporosis. A hunchback is the result of multiple vertebral compression fractures in the spine due to loss of bone mass.

**Loss of height:** Loss of height can also be associated with multiple vertebral compression fractures. Individuals suffering from multiple vertebra compression fractures that result in a kyphotic posture will often suffer a loss of height. Individuals with a loss of more than one or two inches of height are usually the result of compression fractures. Loss of height usually will alert healthcare professionals to further evaluate the risk of osteoporosis.

**Back pain:** Back pain is a common complaint and symptom of many disorders. It is also one of the symptoms of osteoporosis. Individuals with
osteoporosis have been known to have back pain in the lower and upper regions of the back (33).

2.9. CLASSIFICATION OF SYSTEMIC OSTEOPOROSIS

2.9.1. Primary osteoporosis

Primary osteoporosis is the most common type of osteoporosis. It is more common in women than men. A person reaches peak bone mass (density) at about age 30; after that, the rate of bone loss slowly increases, while the rate of bone building decreases. Whether a person develops osteoporosis depends on the thickness of the bones in early life, as well as health, diet, and physical activity at all ages. In women, accelerated bone loss usually begins after monthly menstrual periods stop, when a woman’s production of estrogen slows down (usually between the ages of 45 and 55). In men, gradual bone thinning typically starts at about 45 to 50 years of age, when a man’s production of testosterone slows down. Osteoporosis usually does not have an effect on people until they are 60 or older. Women are usually affected at an earlier age than men, because they start out with lower bone mass(34).
2.9.1.1. Classification of primary osteoporosis

Researchers at Mayo Clinic, U.S.A suggested that primary osteoporosis can be classified as type I or type II osteoporosis (Table 2.1).

Table 2.1 Comparison of type I and type II osteoporosis (35)

<table>
<thead>
<tr>
<th>Factors</th>
<th>Type I</th>
<th>Type II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Postmenopausal Osteoporosis</td>
<td>Age-Related, or Senile, Osteoporosis</td>
</tr>
<tr>
<td>Age</td>
<td>55-75</td>
<td>&gt;70</td>
</tr>
<tr>
<td>Sex (F:M ratio)</td>
<td>6:1</td>
<td>2:1</td>
</tr>
<tr>
<td>Fractures</td>
<td>Wrist and vertebra</td>
<td>Hip and vertebra</td>
</tr>
</tbody>
</table>

2.9.2. Secondary osteoporosis

Secondary osteoporosis results from chronic conditions that contribute significantly to accelerated bone loss. These chronic conditions include thyroxine excess, hyperparathyroidism, malignancies, gastrointestinal diseases, medications, renal failure and connective tissue diseases. Osteoporosis is a common complication of long-term glucocorticoid therapy and is responsive to bisphosphonates in this setting. If secondary osteoporosis is suspected, appropriate diagnostic work-up could identify a different management course. For example, if a pituitary tumor is identified, surgical removal could prevent ongoing accelerated bone loss. The bone loss already sustained can be treated. The secondary hyperparathyroidism of renal failure can be ameliorated through dietary modification and calcium supplementation (36).

2.9.3. Idiopathic juvenile osteoporosis

Idiopathic juvenile osteoporosis is rare. It occurs in children between the ages of 8 and 14 or during times of rapid growth. There is no known cause for this type of osteoporosis, in which there is too little bone formation or excessive bone loss. This condition increases the risk of fractures (35).
2.10. Risk factors of osteoporosis

Many factors that influence risk cannot be changed, such as family history. Other risks can be modified such as stopping smoking. If the risk of osteoporosis can be reduced it may not be needed to perform the bone density test at this time (37).

2.10.1. Risk factors of osteoporosis that cannot be changed or influenced:

2.10.1.1 Being female

Women are at greater risk of osteoporosis as they have smaller bones and hence lower total bone mass. Additionally, women lose bone more quickly following the menopause, and typically live longer. Osteoporosis is less common in men but is still a significant problem. The rate of bone loss in men is less than that in women. In the Framingham osteoporosis study, percent bone loss for women was 0.86% to 1.21% at different sites and for men; 0.04 to 0.90% (38). Secondary causes of osteoporosis are, however, more common in men, affecting approximately 40% of cases (39,40).

2.10.1.2. Advancing age

It is well known that bone mass density decreases with age. Age-related bone mass loss is ascribed to several factors. Nonenzymatic glycation has been proposed as a new potential factor in the loss of bone during aging. A study demonstrates that pentosidine increases exponentially in cortical bone during aging, and is thus a good biomarker for the degree of bone mass density loss. The trabecular bone concentration of pentosidine is more variable, probably because of the turnover rate and the local environment; plasma pentosidine might provide information on the bone turnover rate. The incidence of osteoporosis was 8% and osteopenia 34%. Out of osteoporotic women 31.25% were in the age group 46-50 years and 25% in the age group of 51-55 years (41).
2.10.1.3. Race/ethnicity

Although Caucasian and Asian populations are commonly viewed as high risk groups, Hispanic, American Indians, and African Americans (non-Hispanic black) are at risk of developing osteoporosis (42).

Table 2.2 Estimated percent of adults aged 50 years and over to have osteoporosis or low bone mass by ethnic group, in USA (42)

<table>
<thead>
<tr>
<th></th>
<th>Non-Hispanic White</th>
<th>Asian</th>
<th>Hispanic and American Indians</th>
<th>Non-Hispanic Black</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women with osteoporosis</td>
<td>20%</td>
<td>20%</td>
<td>10%</td>
<td>5%</td>
</tr>
<tr>
<td>Women with low bone mass</td>
<td>52%</td>
<td>52%</td>
<td>49%</td>
<td>35%</td>
</tr>
<tr>
<td>Men with osteoporosis</td>
<td>7%</td>
<td>7%</td>
<td>3%</td>
<td>4%</td>
</tr>
<tr>
<td>Men with low bone mass</td>
<td>35%</td>
<td>35%</td>
<td>23%</td>
<td>19%</td>
</tr>
</tbody>
</table>
2.10.1.4. **Menopause**:

Menopause is a phase in a woman's life, not a disease. Between the ages of 45-55, a woman's ovaries slow down, and in time stop producing an egg every month. A woman's body also begins to make female hormones (estrogen and progesterone) in smaller amounts (43). When Menopause occurring before the age of 45 years is regarded as premature (early onset) menopause. Women who undergo an early menopause, potentially start to lose bone at a significantly earlier age than women who undergo menopause in their fifties. This puts them at a greater risk of developing osteoporosis at an earlier age, than women who undergo menopause at midlife(44).

2.10.1.5. **Family history of osteoporosis**

Lower BMD is found in women and men with a family history of osteoporosis, a family history being defined as a history of osteoporosis or brittle bones, Kyphosis. Individual BMD decreases as the number of family members with osteoporosis increases. Overall family history is a more sensitive predictor of osteoporosis risk than maternal or paternal history alone(45). Prevalence of a positive history in sisters is similar to the prevalence reported for mothers(46).

2.10.1.6. **Amenorrhea**:

This condition typically affects athletes women who do endurance activities or ballet dancers with low body weight and intense exercising. Studies show that women with amenorrhea 20 to 30 percent less bone mineral content than those with regular cycles. The condition is associated with faster bone resorption seen with estrogen deficiency and low body weight (47).

2.10.1.7. **Nulliparity, pregnancy and lactation**

Pregnancy and lactation involve intense physiologic changes that may be important for bone. Both states cause pronounced changes in sex steroids and other hormones involved in calcium homeostasis (48,49), and they also impose calcium losses that could reduce maternal bone mass (49). Indeed, there have been reports of reversible osteoporosis in association with pregnancy. On the other hand, bone loading under pregnancy and the sustained weight gain (48,50,51) that often occurs after delivery have the
potential to increase bone mass. Dietary Calcium absorption becomes more
efficient during pregnancy, a change that also tends to preserve maternal
bone (49). Thus, pregnancy and lactation have the potential to be either
beneficial or detrimental for bone mineral density.

Although nulliparity is an established risk factor for osteoporosis, the
data are conflicting regarding its role in fracture prediction. A study examined
in detail the role of parity in fracture risk; they found that the risk of hip and
vertebral fractures, but not wrist fractures, was significantly higher in
nulliparous women. Of note is that they found that parity had no effect on
BMD. Among nulliparous women, there was a decrease of about 14% ($P =
0.007$) for risk of hip fracture for each additional birth. The authors speculated
that because there was no effect of parity on BMD, pregnancy may exert
some effect on hip geometry (52).

2.10.2. Risk factors of osteoporosis that can be changed or
influenced:

2.10.2.1. Diet

A. Protein

Protein increases bone-formation. Protein intake positively correlates
with both bone-mineral density and hip-fracture incidence; the higher the
average calcium / protein consumption, the more calcium the bones will hold,
on the average, but also the higher the hip-fracture incidence. Excessive
protein intake can also increase excretion of calcium from the body, and some
evidence has linked high-protein diets with osteoporosis, particularly in regard
to animal protein (53). Some people argue that protein even has direct
catabolic effects on bone, due to increased endogenous acid production, and
they point to increased urine calcium levels after animal protein consumption
(54).
B. Sugar

A high-sugar diet may reduce the calcium content of bone. Sugar also causes a significant increase in the fasting serum cortisol levels, and an excess of corticosteroids can cause osteoporosis. It is suggested that a high-sugar diet may reduce the calcium content of bone (55).

C. Alcohol

Alcohol negatively impacts bone health for several reasons; excessive alcohol interferes with the balance of calcium, an essential nutrient for healthy bones, it also increases parathyroid hormone (PTH) levels, which in turn reduce the body’s calcium reserves. Calcium balance is further disrupted by alcohol’s ability to interfere with the production of vitamin D, a vitamin essential for calcium absorption (56).

D. Phosphorus

The increase of phosphorus content up to 1.2-1.8% in the diet (the calcium/phosphorus ratio 1:2 or 1:3) accelerates the development and raises the rate of hypocalcaemia and osteoporosis(57). High phosphorus intakes are also known to suppress the renal synthesis of calcitriol, which in turn could lead to a decrease in calcium absorption. However, there is also some evidence to show that increased phosphorus intake reduces calcium loss and increases absorption of calcium (58).

E. Cola drinks

Middle age and older women may want to limit their consumption of cola-flavored soft drinks. A new study links regular consumption of these beverages with reduced mineral density of hip bones in women past menopause. No similar hip vulnerability to cola showed up in men of the same age. Compared with women who consumed only no cola beverages, those who drank cola more than occasionally had “significantly lower” bone density in their hips, though not in their spines. For instance, drinking one daily serving of cola lowered a woman's bone density about 4 to 5 percent. Whether a woman drank
sweetened or diet cola made no difference. Since men generally have more bone than women to start and so have more calcium to spare (59).

F. Caffeine

Dehydration is one of the main concerns related to caffeine over consumption. Caffeine affects the kidneys by acting as a diuretic, which increases urine production and therefore increases loss of water from the body. Another common concern related to caffeine consumption is its potential effect on bone health. Because caffeine increases urine production, calcium, which is a component of the fluid, is lost. There is some evidence showing that caffeine, and specifically intake of caffeinated beverages, increases the amount of calcium lost in urine. This effect, however, has mainly been observed in postmenopausal women who consumed high amounts of caffeine over time. Most findings suggest that moderate intake is not associated with accelerated bone loss, and that adequate dietary calcium intake can counteract the negative effects of high caffeine consumption (60).

G. Sodium

High sodium levels are closely related to increased calcium excretion. This is a concern, considering that adequate calcium intake plays a major role in reducing the incidence of osteoporosis. It was found that higher levels of sodium predicted higher calcium loss through the urine. Dietary sodium increases urinary calcium leading to a temporary decrease in serum calcium and a consequent rise in PTH. This occurs because in the renal tubules, calcium transport is closely linked to sodium transport and factors that tend to reduce sodium transport at this site, such as high dietary sodium also tend to decrease calcium reabsorption and increase calcium excretion (61).

H. Low dietary calcium intake

Optimal calcium intake is the amount a person needs to reach maximum peak bone mass, maintains adult bone mass, and minimizes bone loss later in life (62). This amount varies throughout a person's lifetime and the National Institutes of Health (NIH) has endorsed new recommendations. Insufficient dietary calcium intake forces hormones such as parathyroid hormone to increase bone resorption. In a normal, healthy diet, dairy products supply about 80% of the daily calcium requirement. Other food sources of
calcium do not contain nearly as much of this element as dairy products do and calcium supplements may be recommended to compensate for dietary deficiency (63).

2.10.2. 2. Smoking

Cigarette smoking was first identified as a risk factor for osteoporosis more than 20 years ago. Recent studies have shown a direct relationship between tobacco use and decreased bone density. Smoking reduces the amount of calcium which bones absorb. Vitamin D helps bones to absorb calcium, but smoking interferes with how the body uses vitamin D. Less calcium is then available to build strong bones. As a result, bones start to get brittle (64). Smoking is known to have systemic antiestrogen effects and other endocrine influences in the body. It is hypothesized that the effects of smoking on bone may begin in adolescence, resulting in lower than normal peak bone mass and smaller bone size (65,66). For women, bone loss occurs rapidly in the perimenopausal and postmenopausal years when endogenous estrogen levels decrease abruptly. Smoking augments this process (66).

2.10.2. 3. lack of exposure to sunlight

The main source of vitamin D is from exposure to sunlight. A study done in Japanese populations shows that elderly people with a low level of activities of daily living (ADL) are at a very high risk of vitamin D insufficiency that deficiency effect on bone metabolism, bone mass, and fractures and make them at high risk having osteoporosis (67).

2.10.2. 4. lack of exercise

Exercise is not just important to general health, it helps build bone mass in youth and slows down bone loss in adults. Exercise is also a factor in helping to reduce the risk of falls as it strengthens muscles, increases flexibility, and improves coordination and balance. During physical activity bones receive messages that they need to work and be strong. When there is a lack of exercise, bones do not receive these messages and lower bone
mass can result. Regular physical activity on a long-term basis maintains the benefits for bone health (68).

2.10. 2. 5. Malnutrition

The importance of malnutrition as a risk factor in osteoporosis is emphasized by the evidence that patients with fractures of the proximal femur are often undernourished. In this study, nutritional status, bone mineral mass and its association with body composition were investigated in underweight and normal weight elderly subjects. Moreover malnutrition in elderly is associated with a higher risk of osteoporosis (69).

2.10.2. 6. Depression

Medical researchers have long known there's a link between depression and lowered bone density, an ailment that can lead to osteoporosis and fractures. Several studies indicate that people with major depression generally have a lower bone mass density compared to control samples (70).

2.10.2. 7. Excess Vitamin A

Retinol is the form of vitamin A of concern. Retinol is commonly found in dietary supplements, fortified foods, and a few animal foods like liver, eggs and dairy products (71). Scientists have found out that too much vitamin A can cause osteoporosis. They speculate that too much vitamin A inhibits bone formation and enhances bone resorption. However, no markers of increased skeletal turnover can be detected accompanying excess vitamin A (72). It appears that vitamin A regulates apoptosis in many different cell lines (71). Retinoic acid also induces cell death of osteoblasts specifically that excessive vitamin A has both differentiating and deteriorial effects on osteoblasts, and that it promotes osteoporosis(73).

2.10.2.8. The effect of corticoids on bone:

Cortisol is a corticosteroid produced and secreted in the body. It is a so-called 'stress hormone. Cortisol-like drugs can cause a loss of bone density, especially amongst postmenopausal women and young men. The bones of the spine and ribs are the most vulnerable to fracturing. Cortisol-like
drugs interfere with the proper functioning of the bone cells and prevent the intestine from properly absorbing calcium. Corticosteroids kill osteoblasts. Killing osteoblasts accelerates the renewal of osteoblasts, and thus accelerates the aging of the capacity of osteoblasts to generate new bone matrix (74).

2.11. Genetic and osteoporosis

There is clear evidence of genetic modulation of bone phenotype parameters including bone density, quantitative ultrasound, bone size, and bone turnover. At any particular age and phase of life, genetic factors explain about 70% of the variance in bone phenotype after adjustment for major medical and disease factors. Hormonal factors, diet, and lifestyle interact with those genetic factors over time.

Common allelic variation in the Vitamin D Receptor (VDR) was the first of several genes and chromosomal loci to be implicated in the genetic determination of bone phenotype. VDR polymorphisms have an effect weaker than originally reported, and part of the allelic effects may be mediated by effects on body size and development and even other hormonal regulators such as PTH or insulin. Irrespective of the strength or mechanism of these associations, these initial findings on the VDR stimulated the field of the genetics of osteoporosis with targeted genetic studies and now genome scan approaches.

Intronic polymorphisms of the collagen Iα1 gene have been shown to be related to bone density and to fracture risk in several studies, although not all findings concur. Common allelic variations have now been associated with bone density for the estrogen receptor, transforming growth factor beta TGFβ receptor, and TGFβ1, for the insulin-like growth factor-I pathway, for interleukin-4 and -6 and the interleukin-1 receptor antagonist, for calcitonin and the PTH receptors and for apolipoprotein E. Of considerable interest, chromosomal loci, notably 11q 12–13, have now been linked to bone phenotypes in human and mouse studies.
Variability of genetic findings across studies seems to be the rule rather than the exception. This variability may relate to interaction of particular loci with specific environmental or even other genetic loci. The importance of genetic heterogeneity, including ethnicity, as well as environmental and hormonal confounders, such as calcium and vitamin D intake, hormonal status and skeletal and body size, will need to be taken into account in future gene search approaches. Genome scans in relation to bone density and fracture endpoints will need to account for such important potential confounders in each target population.

Interactions between genetic and environmental factors, including lifestyle, have been investigated initially for the VDR polymorphisms in relation to the response of bone density and turnover to calcium intake and treatment with simple vitamin D and active vitamin D compounds. Gene-gene and gene-environment interactions in human and animal models will be critical targets for future research. Further genes with positive and negative effects on bone phenotype are certain to be identified in the near future. Each of these will need to be evaluated in relation to potential environmental modulators in pharmacogenetic models. Understanding the molecular physiology of such gene effects is likely to lead to more specific treatments and to allow the selection of more appropriate and effective treatment options (75).

The recent emergence of candidate genes, particularly controlling hormone levels and their receptors, associated with BMD and/or bone remodeling, has opened new concepts in the comprehension of the pathophysiology of osteoporosis. Among these genes, allelic polymorphisms for the receptors recognizing the vitamin D active metabolite (calcitriol), estradiol, and PTH could be associated with bone mass. Most importantly, these polymorphic genes, as well as those coding for TGF-β and IL-6, have a potential interest to understand, and eventually predict, the individual response to anti-osteoporotic therapies. Thus, the possible implication of the PTH receptor 1 gene in the response to PTH raises great interest with respect to the recently reported effects of PTH in the prevention of fracture risk (76).
2.12. Epidemiology of osteoporosis

Osteoporotic fractures represent a significant public health burden, which is set to increase in future generations. Lifetime risk is high and lies within the range of 40% to 50% in women and 13% to 22% in men. Life expectancy is increasing worldwide, and it is estimated that the number of individuals aged 65 years and older will increase from 323 million to 1555 million by the year 2050. These demographic changes alone can be expected to cause the number of hip fractures occurring worldwide to increase from 1.66 million in 1990 to 6.26 million in 2050. Based on current trends, hip fracture rates might increase in the United Kingdom from 46,000 in 1985 to 117,000 in 2016 (77).

**Prevalence of osteoporosis**

**Table 2.3 world wide prevalence rate for osteoporosis (14,15)**

<table>
<thead>
<tr>
<th>Country/Region</th>
<th>Extrapolated Prevalence</th>
<th>Population Estimated Used</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Osteoporosis in North America (Extrapolated Statistics)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>USA</td>
<td>30,229,233</td>
<td>293,655,405</td>
</tr>
<tr>
<td>Canada</td>
<td>3,346,398</td>
<td>32,507,874</td>
</tr>
<tr>
<td><strong>Osteoporosis in Europe (Extrapolated Statistics)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Austria</td>
<td>841,519</td>
<td>8,174,762</td>
</tr>
<tr>
<td>Britain (United Kingdom)</td>
<td>6,204,337</td>
<td>60,270,708 for UK</td>
</tr>
<tr>
<td>France</td>
<td>6,220,139</td>
<td>60,424,213</td>
</tr>
<tr>
<td>Germany</td>
<td>8,484,886</td>
<td>82,424,609</td>
</tr>
<tr>
<td>Italy</td>
<td>5,976,505</td>
<td>58,057,477</td>
</tr>
<tr>
<td>Poland</td>
<td>3,976,241</td>
<td>38,626,349</td>
</tr>
<tr>
<td><strong>Osteoporosis in Asia (Extrapolated Statistics)</strong></td>
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<td></td>
</tr>
<tr>
<td>Japan</td>
<td>13,107,809</td>
<td>127,333,002</td>
</tr>
<tr>
<td>Pakistan</td>
<td>16,387,858</td>
<td>159,196,336</td>
</tr>
<tr>
<td>North Korea</td>
<td>2,336,512</td>
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<tr>
<td>South Korea</td>
<td>4,965,240</td>
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<td><strong>Osteoporosis in Eastern Europe (Extrapolated Statistics)</strong></td>
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<tr>
<td>Russia</td>
<td>14,820,859</td>
<td>143,974,059</td>
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<td><strong>Osteoporosis in Australasia and Southern Pacific (Extrapolated Statistics)</strong></td>
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<tr>
<td>Australia</td>
<td>2,049,882</td>
<td>19,913,144</td>
</tr>
<tr>
<td><strong>Osteoporosis in the Middle East (Extrapolated Statistics)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Afghanistan</td>
<td>2,935,231</td>
<td>28,513,677</td>
</tr>
<tr>
<td>Country</td>
<td>Population</td>
<td>Total Population</td>
</tr>
<tr>
<td>------------------</td>
<td>------------</td>
<td>------------------</td>
</tr>
<tr>
<td>Egypt</td>
<td>7,835,617</td>
<td>76,117,421</td>
</tr>
<tr>
<td>Gaza strip</td>
<td>136,396</td>
<td>1,324,991</td>
</tr>
<tr>
<td>Iran</td>
<td>6,948,859</td>
<td>67,503,205</td>
</tr>
<tr>
<td>Iraq</td>
<td>2,612,100</td>
<td>25,374,691</td>
</tr>
<tr>
<td>Israel</td>
<td>638,133</td>
<td>6,199,008</td>
</tr>
<tr>
<td>Jordan</td>
<td>577,623</td>
<td>5,611,202</td>
</tr>
<tr>
<td>Kuwait</td>
<td>232,394</td>
<td>2,257,549</td>
</tr>
<tr>
<td>Lebanon</td>
<td>388,831</td>
<td>3,777,218</td>
</tr>
<tr>
<td>Libya</td>
<td>579,722</td>
<td>5,631,585</td>
</tr>
<tr>
<td>Saudi Arabia</td>
<td>2,655,464</td>
<td>25,795,938</td>
</tr>
<tr>
<td>Syria</td>
<td>1,854,678</td>
<td>18,016,874</td>
</tr>
<tr>
<td>Turkey</td>
<td>7,092,021</td>
<td>68,893,918</td>
</tr>
<tr>
<td>United Arab Emirates</td>
<td>259,814</td>
<td>2,523,915</td>
</tr>
<tr>
<td>West Bank</td>
<td>237,918</td>
<td>2,311,204</td>
</tr>
<tr>
<td>Yemen</td>
<td>2,061,383</td>
<td>20,024,867</td>
</tr>
<tr>
<td>Sudan</td>
<td>4,029,957</td>
<td>39,148,162</td>
</tr>
<tr>
<td>South Africa</td>
<td>4,575,577</td>
<td>44,448,470</td>
</tr>
</tbody>
</table>

2.13. Menopause

2.13.1 Definition:

The term "menopause" comes from two Greek words that mean "month" and "to end". In other words, it translates as "the end of the monthlies." Menopause begins when a woman's level of the hormone estrogen falls to a very low level and the menstrual cycle stops. The average age for menopause is 51 (78).
2.13.2. Stages of menopause

2.13.2.1. Perimenopause

Perimenopause is the time leading up to menopause when you start to notice menopause-related changes plus the year after menopause. Perimenopause is what some people call "being in menopause" or "going through menopause." But menopause itself is the day when women haven’t had a period for 12 months in a row. During perimenopause, ovaries start to shut down, making less of certain hormones (estrogen and progesterone), and begin to lose the ability to become pregnant (79).

2.13.2.2 Menopause:

Menopause is defined as a cessation of the menses for more than 6 months. Menopause results from a loss of oocytes and a dropping ovarian response to the trophic peptide hormones of the pituitary gland. Thus, no matter how much FSH and LH are secreted by the pituitary gland, the ovary continues to deteriorate. The end stages of ovarian failure are documented by a male range of estrogen values, no ovulation, and thus no progesterone, and markedly elevated FSH and LH. The ovarian deterioration occurs over 1 year to 18 months (80).

2.13.2.3. Postmenopause:

The postmenopausal phase of a woman's life begins at menopause, which is 1 year after her last menstrual period. While postmenopause usually begins around age 50, some women become postmenopausal in their mid-40s, and others do so in their later 50s. In early postmenopause, a woman's estrogen stabilizes at a low level (81).

2.13.3. Types of menopause:

2.13.3.1. Premature menopause:

The term premature ovarian failure describes a stop in the normal functioning of the ovaries in a woman younger than age 40. Some people also use the term primary ovarian insufficiency to describe this condition. In menopause, a woman will likely never have another menstrual period again; women with premature ovarian failure are much more likely to get periods,
even if they come irregularly. A woman in menopause has virtually no chance of getting pregnant; a woman with premature ovarian failure has a greatly reduced chance of getting pregnant, but pregnancy is still possible (82). Although the exact cause of premature ovarian failure may be unknown; a genetic factor autoimmune disease, chemotherapy, radiation therapy related, or hysterectomy are among the causes of premature menopause (83).

2.13.3.2. Surgical menopause

Surgical menopause is the removal of both ovaries in women who have not yet had natural menopause. It almost always occurs with hysterectomy (removal of the uterus). Surgical menopause occurs very suddenly; one day a woman is having menstrual cycles, and the next day, after surgery, she is postmenopausal. Women with natural menopause have a gradual transition that can take many years. Women with surgical menopause often experience more intensity in their symptoms than women with natural menopause. Women with surgical menopause are younger than women with natural menopause. They have to heal both physically and mentally to adjust to what happened (84).

2.13.3.3. Natural menopause

Natural menopause occurs when the ovaries naturally decrease their production of the sex hormones estrogen and progesterone; there are no menstrual periods for 12 consecutive months (85).

2.13.4. Symptoms of menopause

Menopause symptoms begin gradually while the ovaries are still functioning and a woman is still having menstrual periods. These symptoms can begin as early as the 4th decade of life (when a woman is in her 30s) and may persist for years until menopause has occurred. The Menopause symptoms can be perceived as physical problems; irregular vaginal bleeding, hot flashes and night sweats and emotional disturbances; mood changes, anxiety, forgetfulness or problems with focus and concentration (86).
2.13.5. Causes of menopause

The menopause happens when the ovaries stop responding to certain hormones from the brain, and so eggs stop maturing regularly. There is a drop in the levels of estrogen and progesterone (the two female sex hormones produced by the ovaries). It is this fall in hormone levels that causes symptoms of menopause (87).

2.13.6. Level of estrogen in women life

Estrogen levels play an important role in a woman's life. They rise at puberty with the onset of childbearing years and then increase and decrease rhythmically with menstrual cycle. They peak during pregnancy and then decline as reaching menopause and menstrual cycle ceases. Figure 2.3 shows estrogen levels during women life. This decline in estrogen levels during menopause gives rise to common symptoms such as hot flashes and night sweats (88).

![Figure 2.3 Estrogen levels during women life (88).](image)
2.13. 7. Global statistics of women at menopause

In 1990, the total population of postmenopausal women throughout the world was reported to number 476 million. An analysis of the distribution of postmenopausal women revealed that 40% live in the industrialized world. It is predicted that the total number of postmenopausal women in 2030 will be approximately 1200 million and the proportion of those living in the developing world will increase to 76% [89]. The available data from 1989 to 1992 postulated that the percentages of the population above 45 years of age in the 11 countries (except Japan) in the East Asian region ranged from 15.3 to 24.0%, and those above 65 ranged from 3.8 to 6.6% [90]. In Japan, the proportion of the population above 65 years of age in 1995 was 14.5% and the male: female ratio was 2:3. It is projected that the proportion will increase up to 27% in 2025 [91]. The population projections for China given in the 1993 World Development Report [89] indicated that the proportion of women aged 50 and over to the total population in 1990 was 8.20% and that in 2010 and 2030 it will be 11.28 and 17.08%, respectively.

2.14. Diagnosis of osteoporosis in menopause women:

The diagnosis of osteoporosis is usually made by the doctor using a combination of complete medical history, physical examination, laboratory tests, skeletal x-rays and bone densitometry (a bone density scan). If the doctor finds low bone mass, he may want to perform additional tests to rule out the possibility of other diseases that can cause bone loss, such as a vitamin D deficiency (osteomalacia) or overactive parathyroid glands (hyperparathyroidism) [92].

2.14.1. History and physical examination

An osteoporosis examination includes a number of questions to assess risk factors. These include family history, history of fractures, menstrual history, dietary history, medications, habits such as cigarette smoking and alcohol consumption, and a review of past medical history and other medical conditions. The physical exam includes a measurement of height, an assessment of spinal tenderness and curvature, and a search for signs of other medical conditions that may contribute to osteoporosis [93].
2.14.2. Bone mineral density

A bone mineral density test (BMD), a non-invasive and painless test, is the best way to determine bone health. BMD tests can identify osteoporosis, determine the risk for fractures and monitor the response to an osteoporosis treatment. Different BMD tests may measure the hip, spine, wrist, finger, shin bone or heel (94). The National Osteoporosis Foundation recommends BMD testing for the following individuals (95):

- All women aged 65 and older regardless of risk factors
- Younger postmenopausal women with one or more risk factors.
- Postmenopausal women who present with fractures (to confirm the diagnosis and determine disease severity).
- Estrogen deficient women at clinical risk for osteoporosis.
- Individuals with vertebral abnormalities.
- Individuals receiving, or planning to receive, long-term glucocorticoids (steroid) therapy.
- Individuals with primary hyperparathyroidism.
- Individuals being monitored to assess the response or efficacy of an approved osteoporosis drug therapy.

2.14.3. Common bone mineral density tests

- Dual Energy X-ray Absorptiometry (DEXA).
- Peripheral Dual Energy X-ray Absorptiometry(pDXA).
- Single Energy X-ray Absorptiometry(SXA)
- Peripheral Quantitative Computed Tomography(pQCT).
- Radiographic Absorptiometry(RA).
- Quantitative Computed Tomography(QCT).
- Quantitative Ultrasound (QUS) (96).
2.14.4. Various BMD tests

Characteristics of various BMD tests are shown in Table 2.4 and evaluation of three of them is shown in Table 2.5.

Table 2.4 Tests to evaluate bone health (97)

<table>
<thead>
<tr>
<th>Type of Test</th>
<th>How It Is Done</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEXA</td>
<td>A scan is taken of body</td>
<td>The gold standard for bone density; it measures density of spine and hip and establishes a diagnosis of osteoporosis</td>
</tr>
<tr>
<td>PDXA</td>
<td>A scan is taken of the wrist, finger, or heel</td>
<td>Bone density at specific site tested; does not necessarily correspond to hip or spine</td>
</tr>
<tr>
<td>QUS (Quantitative Ultrasound)</td>
<td>An ultrasound scan is taken of the heel and shin</td>
<td>Determines bone density of the heel, which corresponds closely to the hip. Used as a convenient, quick screening tool</td>
</tr>
<tr>
<td>X-ray</td>
<td>A picture of bones is taken</td>
<td>Evaluates fractures and other bone problems; not a screening tool for osteoporosis</td>
</tr>
<tr>
<td>Bone scan</td>
<td>Dye is injected into blood; hours later, a scan taken</td>
<td>Evaluates a possible stress fracture or other problem with the bone; not a screening tool for osteoporosis</td>
</tr>
</tbody>
</table>

Table 2.5. Comparison of different modalities for assessing bone fracture risk (98)

<table>
<thead>
<tr>
<th>Factor</th>
<th>DXA</th>
<th>QCT</th>
<th>US</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost</td>
<td>Intermediate</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Radiation</td>
<td>Low</td>
<td>High</td>
<td>None</td>
</tr>
<tr>
<td>Portability</td>
<td>Limited</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Parts measured</td>
<td>Spine, hip, wrist</td>
<td>Spine, hip</td>
<td>Calcaneus</td>
</tr>
<tr>
<td>Precision</td>
<td>Excellent</td>
<td>Good</td>
<td>Low</td>
</tr>
<tr>
<td>Monitoring of treatment response</td>
<td>Excellent</td>
<td>Good</td>
<td>Low</td>
</tr>
</tbody>
</table>

DXA = Dual-energy x-Ray Absorptiometry; QCT = Quantitative Computed Tomography; US = Ultrasonography.
2.14.5. Interpretation of BMD results

Table 2.6 contains the World Health Organization’s definitions of osteoporosis based on bone mineral density.

**Table 2.6. Criteria for defining bone density "T-scores" (99)**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>BMD value within 1 SD of the young adult reference mean (T &gt;=-1.0)</td>
</tr>
<tr>
<td>Osteopenia</td>
<td>BMD value of more than 1 SD below the young adult mean but less than 2.5 SD below this value (-1.0 &gt; T &gt; -2.5)</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>BMD value of 2.5 SD or more below the adult mean value (T &lt;=-2.5)</td>
</tr>
<tr>
<td>Established osteoporosis</td>
<td>BMD value of 2.5 SD or more below the adult mean value (T &lt;=-2.5) in the presence of one or more fragility fractures</td>
</tr>
</tbody>
</table>

* SD: Standard Deviation

**Z-score**

BMD value may also be compared to other people of your age, sex, and race. This is called Z-score. It is given in standard deviations (SD) from the average value for age group.

- A negative (−) value means that bones are thinner (lower bone density) and weaker than most people in the same age group. The more negative the number is, the less the bone density compared with others in the same age group.
- A positive (+) value means that bones are thicker and stronger than most people in the same age group (99).
2.14.6. Clinical laboratory investigations

Over the last few years biochemical markers of bone turnover have been developed. By measuring the breakdown products of bone in urine, we can now identify those at risk of losing bone through high bone turnover. These assays are now widely available and have the following applications: Identification of people with high bone turnover and therefore at potential risk of developing osteoporosis later in life, improved use of drug therapies and reduction in costs by targeting therapy more effectively, improved compliance for long term therapies by regular monitoring and better targeting of DEXA resources. The use of biochemical tests to identify patients with accelerated bone loss should be available to all primary care sectors. Patients prescribed hormone replacement therapy (HRT), bisphosphonates or other therapies should be monitored using biochemical tests in order to ensure compliance and therapeutic effect (100).

2.14.7. The most common laboratory tests

- Blood calcium levels
- Blood vitamin D levels
- Thyroid function
- Parathyroid hormone levels
- Estradiol levels
- Follicle stimulating hormone (FSH) test to establish menopause status
- Osteocalcin levels to measure bone formation.
  - Blood phosphorus levels
  - Blood magnesium levels
  - Blood sodium levels (100).
  - A 24-hour urinary calcium study (101).

2.14.8. Bone markers

2.14.8.1 Osteocalcin

Osteocalcin also called as Gla protein, is marker of bone formation. It is vitamin K and vitamin D-dependent protein produced by osteoblasts and is the most abundant and most widely studied of the non-collagenous proteins in bone. (102,103). Osteocalcin is more sensitive marker than serum alkaline phosphatase (104). It has been suggested that there are discrete groups of postmenopausal
osteoporotic women, with normal, high or low bone formation (105). In support of this, osteocalcin concentration have been reported as similar, higher or lower than normal age matched controls (106,107). An elevation of 10% has been reported in the mean osteocalcin concentration in postmenopausal osteoporotic women, in contrast to the greater elevation reported in bone resorption markers (108). Serum osteocalcin concentration correlate with the rate of bone loss from the distal forearm (109). However, the overlap of the results is too great for the diagnosis of osteoporosis in individuals and scatter around the regression line is too great to identify individuals with accelerated bone loss. Monitoring osteocalcin concentration may be useful in determining the response to treatment for metabolic bone disease or the prediction of the bone loss in postmenopausal women (110).

2.14.8. 2 Alkaline phosphatase

There was a slight but significant negative correlation indicating an increasing alkaline phosphatase activity with decreasing bone mass. This correlation was not caused by interaction of age. The changes could not be explained by fractures. It is suggested that a slight increase in the alkaline phosphatase activity in women with a more severe osteoporosis is related to bone resorption (111).

2.14.8. 3. Magnesium

Bone health is supported by many factors, most notably calcium and vitamin D. However, some evidence suggests that magnesium deficiency may be an additional risk factor for postmenopausal osteoporosis. This may be due to the fact that magnesium deficiency alters calcium metabolism and the hormones that regulate calcium. Decreased serum magnesium levels have been found in postmenopausal patients with osteoporosis. Magnesium testing can be used effectively to help identify a woman’s risk of osteoporosis. Thus, standard magnesium testing is encouraged for all postmenopausal women (112).
2.14.8. 4 Blood calcium and phosphorus levels

The levels of both Ca and P are usually normal in osteoporosis but may be elevated with other bone diseases(113).

2.14.8. 5 Estradiol levels

In women, serum estradiol is an important determinant of bone loss; when ovarian estrogen production decreases and serum levels fall into the postmenopausal range (<30 pg/mL), accelerated bone loss ensues (114). Estrogen replacement therapy typically elevates serum estradiol levels to the range of 40–60 pg/mL (115), and these levels are considered the minimum level sufficient to prevent or retard bone loss (116).

2.15. Basic prevention:

There are steps that can be taken to prevent osteoporosis which include:

2.15. 1. Diet:

2.15. 1.1. Calcium intake:

A good calcium intake is essential throughout life for healthy bones. There is good evidence that the adequacy of a child’s diet at least partially determines their osteoporosis risk in adulthood.

Calcium Supplements

Two of the most common forms of calcium supplements are calcium citrate and calcium carbonate.

Calcium citrate is absorbed best by our bodies and can be taken any time. Calcium citrate is often recommended for the older population because as we get older, we produce less gastric acid and gastric acidity is not needed for it to be absorbed.

Calcium carbonate is another good choice. It is best absorbed when taken with food, as gastric acid is needed for it to be absorbed (117).

2.15. 1. 2. Vitamin D:

Vitamin D is found in numerous dietary sources such as fish, eggs, fortified milk, and cod liver oil. The sun is also a significant contributor to our daily production of vitamin D, and as little as 10 minutes of exposure is
thought to be enough to prevent deficiencies. The term "vitamin D" refers to several different forms of this vitamin. Two forms are important in humans: ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3). Vitamin D2 is synthesized by plants. Vitamin D3 is synthesized by humans in the skin when it is exposed to ultraviolet-B (UVB) rays from sunlight or the diet. The major biologic function of vitamin D is to maintain normal blood levels of calcium and phosphorus. Vitamin D aids in the absorption of calcium, helping to form and maintain strong bones. Recently, research also suggests vitamin D may provide protection from osteoporosis, hypertension, cancer, and several autoimmune diseases (118).

2.15. 1.3. Soybeans in prevention of osteoporosis

Soybeans are a unique source of a group of compounds called isoflavones. Soybeans are the only food that contain these compounds in significant amounts. One type of isoflavone called daidzein is very similar to a drug widely used in Asia and Europe to treat osteoporosis. This drug prevents bone from breaking down. When the drug is metabolized in the body, it produces daidzein - the same compound found in soybeans. This suggests that eating soyfoods - natural sources of daidzein - could help reduce the risk of osteoporosis. Another isoflavone in soyfoods, genistein, was shown to inhibit breakdown of bone (119).

2.15. 1.4. Onion in prevention of osteoporosis

In a current study, the researchers analyzed the active chemical components of white onions and found that the most likely compound responsible for the decreased bone loss was a glutamyl peptide called GPCS. The researchers then obtained a group of isolated bone cells from newborn rats and exposed the cells to parathyroid hormone to stimulate bone loss, then exposed some of the treated cells to GPCS. Treatment with GPCS significantly inhibited the loss of bone minerals, including calcium, when compared to cells that were not exposed to GPCS. Additional studies are needed to determine whether GPCS will have a similar effect in people, how much onion or GPCS is needed for a positive effect on bone health, and to determine the mechanism of action of GPCS on bone cells (120).
2.15. 1.5. **Green tea**

Green tea is loaded with catechin polyphenols, especially epigallocatecchin gallate, which is commonly referred to as EGCG. Consumption of green tea, a rich source of EGCG, is associated with increased bone mineral density. The observed effects of EGCG on bone formation suggest that EGCG may have beneficial effects on bone health and also acts as an antioxidant (121).

2.15. 1.6. **Limit caffeine, protein, vitamin A, and sodium**

The amount of caffeine in a single cup of brewed coffee causes a small reduction in calcium absorption, which can be easily offset by adding a tablespoon or two of milk. Moderate intake of caffeine is considered to be 300 mg/day, which is about the amount in 3 cups of coffee to reduce the effect of it on bone metabolism (122). Experimentally, diets high in protein can induce a negative calcium balance by increasing urinary loss of calcium, which could lead to bone loss if calcium absorption is not up-regulated. This overshadows the basic principle that protein is needed for bone formation. Sodium (and chloride), the components of table salt, increase the calcium requirement by increasing urinary calcium excretion (123). Studies done in Ireland area involving animal models and case reports have documented that hypervitaminosis A is associated with bone resorption, hypercalcaemia and bone abnormalities. More recently, some epidemiological studies have suggested that high habitual intake of vitamin A could contribute to low bone mineral content and fracture risk so limiting vitamin A intake reduce the risk of having fractures (124).

2. 15.2. **Exercise**

Weight-bearing exercise is vital for bone health. Running, jogging, walking, dancing, and weight training are all exercises that put more weight on bones than activities such as swimming. The added weight on the legs encourages bone formation, creating a stronger frame that has less chance of being fractured. Exercise also decreases the risk of falls by improving balance. A person should begin with a simple exercise protocol from a qualified health care professional (125).
2.15.3. Preventing falls

Osteoporotic fractures, particularly of the long bones, result from falls, and most such falls occur during usual daily activities. There is now solid evidence that more than a third of these falls and the injuries resulting from them could be prevented by interventions that address specific risk factors. Falls have been prevented by reducing the use of psychotropic medications, reducing the use of all medications, correcting postural hypotension and the cardiovascular causes of syncope, offering advice on home modification and safety, and prescribing specific exercises to improve strength and balance (126).

2.16. Drugs for the treatment of osteoporosis

Any person diagnosed with osteoporosis, and even if they have had fractures, it’s never too late to begin treatment. Besides stopping further bone loss, some newer drugs can even improve bone strength. Medications available for treating osteoporosis include: Bisphosphonates, Selective Estrogen Receptor Modulators (SERMs), Raloxifene, Calcium Supplements, Vitamin D, Parathyroid Hormone, Strontium Ranelate, Ibandronate Sodium (127).

2.17. Previous study

A study done in United Arab Emirates reported that the majority of women (67.9%) didn’t have a family history of osteoporosis. Seventeen percent were unsure of whether they had a family history of osteoporosis and 57% of women never avoid dairy products. Also, the majority of women drink 2 cups and more of coffee or tea a day. Moreover, this result showed that 60% of women preferred walking as a type of exercise. It was found that 30.2% of the study population suffered from osteoporosis, approximately 23% of women are taking calcium supplements and 5.7% of women are taking hormone replacement therapy to treat this disease. Most of the women (83%) had never had a bone fracture and the majority of women (50.9%) would classify their activity as light. Twenty four percent of women didn’t do physical activity (128).
Study was carried out at Saudi Arabia found that the higher prevalence of osteoporosis could be explained to the similar risk factors including Vitamin D deficiency, multiparty, nonexposure to sun, lack of exercise and poor intake of dairy products rich in vitamin D(129).

study done in Turkish area to measure serum osteocalcin (OSC), calcium (Ca), alkaline phosphatase (ALP) that to investigate the relationship of these parameters with bone mineral density (BMD) and bone turnover markers in postmenopausal women with and without osteoporosis. The values of OSC levels were significantly higher in postmenopausal osteoporotic than in nonosteoporotic women (P < 0.05). while activity of calcium (Ca), alkaline phosphatase (ALP) was slightly higher in postmenopausal osteoporotic women than in postmenopausal nonosteoporotic women, but the difference did not reach a statistical significance (130).

study done by Jasminka Z et al (2000) in USA reported that, there is a positive relationship between sodium intake and urinary Ca excretion. Na excretion in a cross-sectional study of 440 healthy postmenopausal women, Results show strong correlation between urinary Na and Ca (131).
Chapter Three

Materials and Methods
Material and Methods

3.1 Study design
   Case control study.

3.2. Study population
   3.2.1. patients group
   This group consisted of 96 menopausal women (40-60 years) and their mean age was 45.6 and were diagnosed as osteoporotic. Informed consent was obtained from all women enrolled in the study.

   3.2.2. controls group
   A group of 96 healthy women with age of 40-60 years and their mean age was 53.79 were enrolled in the study. All subjects were at menopause were not diagnosed as suffering from osteoporosis.

3.3. Selection of subjects
   All patients with osteoporosis (admitted to the bone outpatient clinic of the two main hospitals in Gaza Strip: Al-Shifa, European hospitals. And matched the inclusion criteria (shown below) were set by the researcher in the patients group.
   All healthy subjects have no records of osteoporosis and matched the inclusion criteria were set by the researcher in the control group.
   Information regarding the subjects were obtained through a structured questionnaire (closed ended questions). Subjects interview was done at the three main hospitals.
   Questionnaire information included age, height, weight, risk factors and used medications.

3.4. Questionnaire interview
   A group of 10 healthy subjects were recruited in the pilot study to optimize laboratory techniques and examine validity of the questionnaire.

3.5. Inclusion criteria
   • Patients group patient subjects included in this group were those who met the following requirements:
     _ Hospital admission or visit bone clinic and diagnosed as osteoporotic.
     _ 40-60 years old.
- At menopause
  
  - **Control group** healthy subjects included in this group were those who met the following requirements:
    - Had no records of osteoporosis.
    - 40-60 years old.
    - At menopause.

3.6. **Subject identification**

All patients who met the inclusion criteria were given a numerical codes. The study location was identified in each hospital and department. The subject serial number identified the subject and her study documents. The researcher kept a list identifying the names of the subjects their respective serial number and dates of sample collection until the completion of the study to allow checking data if needed.

3.7. **Ethical considerations**

The study protocols was approved by the local ethics committee (Palestinian National Authority, MOH, Helsinki Committee).

3.8. **Materials and reagents**

1. Sterile syringes and needles.
2. Alcohol.
3. Red top tubes for serum.
4. Adhesive tape
5. Ice box for keeping the sample through transportation.
7. Human Osteocalcin ELISA kits
8. Magnesium XL FS kits. (Diasys Diagnostic Systems GmbH)
9. Alkaline phosphatase FS kits. (Diasys Diagnostic Systems GmbH)
10. Calcium CPC FS kits. (Diasys Diagnostic Systems GmbH)
11. Phosphate FS kits. (Diasys Diagnostic Systems GmbH)
12. Sodium reagent set. (Diasys Diagnostic Systems GmbH)
13. Estradiol FS kits. (Diasys Diagnostic Systems GmbH)
14. NaCl solution
15. Microtitration plate ELISA reader capable of absorbance measurement at 450 nm
16. Deionized water
17. Precision pipette to deliver 10 µl, 100 µl, 220 µl, and 1.0 mL.
18. Semi-automatic pipette to deliver 100 µl
Microtitration plate shaker capable of 500-700 orbital revolution per minute
19. Automatic microtitration plate washer
20. Vortex mixer
21. Absorbent materials for bloting the strips
22. Graph paper for manual data reduction
23. Disposable 12×75 mm glass tubes

3.9. Instruments
1. Diamed Elisa Reader.
2. Rayto Reader.
3. Ecolyte Reader.
5. Test tubes/rack.

3.9. Blood sampling and processing
• Blood samples for testing osteoporosis were collected overnight fasting.
• Five ml venous blood were drawn in red top tubes for serum by researcher.
• Serum samples were rapidly separated within 3 hours from collection by centrifugation at 3500rpm for 10 min at room temperature, separated into three tubes one for chemistry, another for estradiol test and last one for osteocalcin.
• Samples were stored in tubes at -20 C (samples in this way are stable for up to 3 months).
• The samples were analyzed in private laboratory in Gaza.

3.10.1. Osteocalcin
• Principle of test
The DSL Osteocalcin assay is an enzymatic amplified “one-step” sandwich-type immunoassay. In the assay standards, controls and unknown diluted serum samples were incubated with anti-osteocalcin polyclonal detection antibody labeled with the enzyme horseradish peroxidase in microtitration wells coated with an affinity purified anti-osteocalcin mouse monoclonal antibody. After incubation and washing, the wells were incubated with the substrate tetramethybenzidine (TMB). An acidic stopping solution was then added and the degree of enzymatic turnover of the substrate was determined by dual wavelength absorbance measurement at 450 nm.
The absorbance was directly proportional to the serum concentration of osteocalcin. A set of osteocalcin standards were used to plot a standard curve of absorbance versus osteocalcin concentration from which osteocalcin of the unknown samples could be calculated.

- **Reagents supplied**
  
  The DSL -10-7600 Active Human Osteocalcin Elisa Kit contains sufficient reagents for 96 wells. Each kit contains the following reagents:
  
  a. **Anti-osteocalcin coated microtitration strips**:

  The stripholder containing 96 Microtitration wells. Coated with anti-Osteocalcin monoclonal antibody. Stored at 2-8º C.

  b. **Sample diluent**

  One bottle, 55 ml, containing 0 ng/ ml osteocalcin in protein based buffer with a non-mercury preservation. stored at 2-8º C.

  c. **Osteocalcin standard (Lyophilized)**

  Five vials, labeled B-F, containing approximate concentration of 5, 25, 65, 125 and 250 ng/ml osteocalcin (synthetic 1-49) in a protein –based buffer with a non-mercury preservation. Standards were reconstituted B-F with 1.0 ml deionized water.

  d. **Osteocalcin controls : (Lyophilized)**

  Two vials, 1 ml each, Levels 1 and 2, containing low and high concentration of osteocalcin in protein based buffer with a non-mercury preservation.

  e. **Osteocalcin assay buffer**:

  One amber vial, 0.3 ml, containing affinity purified anti-osteocalcin polyclonal antibody conjugated to the enzyme horseradish peroxidase in protein –based buffer with a non-mercury preservation. Diluted just prior to use in the osteocalcin assay buffer. Stored at 2-8º C until expiration date.

  Note : the dilution of this reagent was made 10-15 minutes before its use in the assay.

  g. **TMB Chromogen Solution**:

  One amber, 11 ml, containing a solution of tetramethylbenzidine (TMB) in citrate buffer with hydrogen peroxide. Stored at 2-8º C.

  h. **Wash Concentrate**:

  One bottle, 60 ml, containing buffered saline with a nonionic detergent. Diluted 25 – fold with deionized water prior to use. Stored at (25º C) or 2-8 ºC.
I. Stopping solution:
One vial, 11 ml, containing 0.2 M sulfuric acid. Stored at 2-8 °C until expiration date.

Note: All reagents and samples were allowed to reach room temperature (25º C) and mixed thoroughly by gentle inversion before use.

• Specimen collection and preparation
Clear, non hemolyzed or lipemic serum was used and the usual precautions for venipuncture were followed.

Test procedure:
C. Assay procedure
All specimens and reagents were allowed to reach room temperature before use. After reconstitution of standards and controls, mixed thoroughly, avoiding foam, before use. Assay standards, controls and unknowns.

1. Mark the microtitration strips to be used.
2. Pipet 100 µL of each standards, controls and unknown to the appropriate well.
3. The Anti-enzyme conjugate was prepared by diluting the conjugate with assay buffer.
4. Add 100 µL of the antibody enzyme conjugate solution to each well using a semi-automatic dispenser.
5. The wells were incubated and shaked at fast speed on an orbital microplate shaker for 2 hours at room temperature 25ºC.
6. Each well was aspirated and washed each well five times with the washing solution using an automatic microplate washer. Plates were on blot dry were absorbent material.
7. Add 100 µL of the (TMB) chromogen solution to each well using a semi-automatic dispenser.
8. The well was incubated, shaked at fast speed on an orbital microplate shaker for 10 minutes at room temperature 25ºC. Avoided exposure to direct sunlight.
9. 100 µl was added as stopping solution to each well using a semi-automatic dispenser.
10. The solution in the wells was readed within 30 minutes, using microplate reader set to 450 nm.
3.10.2 Calcium

**Principle of the test**
Cresolphthalien complexone reacts with calcium ions in alkaline medium forming a red–violet color. Interference by magnesium is eliminated by addition of 8-hydroxy-quinoline.

**Method**
Photometric test using Cresolphthalien complexone

**Reagents**

| R 1: Ethanolamine detergents          | pH 10.7 600 mmol/l |
| R 2: 2- Cresolphthalien complexone   | 0.06 mmol/l       |
| 8-hydroxy-quinoline                  | 7 mmol/l          |
| Hydrochloric acid pH 1.1              | 20 mmol/l         |

**Standard**: 10 mg/dl (2.5mmol/l)

**Specimen**: serum.

**Assay procedure**

Wavelength: 570 nm

Optical path: 1 cm

Temperature: room temperature

Measurement: against reagent blank

**Substrate start**

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Sample or standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample or standard</td>
<td>-</td>
<td>20 µl</td>
</tr>
<tr>
<td>Dist. water</td>
<td>20 µl</td>
<td>-</td>
</tr>
<tr>
<td>Reagent 1</td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
<tr>
<td>Mix and read absorbance A1 after 5-30 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reagent 2</td>
<td>250 µl</td>
<td>250 µl</td>
</tr>
<tr>
<td>Mix, read absorbance A2 after 5-30 min</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Sample start

<table>
<thead>
<tr>
<th>Blank</th>
<th>Sample or standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample or standard</td>
<td>-</td>
</tr>
<tr>
<td>Dist. water</td>
<td>20 µl</td>
</tr>
<tr>
<td>Monoreagent</td>
<td>1000 µl</td>
</tr>
<tr>
<td>Mix and read absorbance after 5-30 min against</td>
<td></td>
</tr>
</tbody>
</table>

### Reference range
Serum: 8.6-10.3 mg/dl (2.15-2.5 mmol/l).

### 3.10.3 Phosphate

#### Principle of the test
Ammonium molybdate + sulphuric acid + Phosphate $\rightarrow$ Inorganic phosphorus molybdate complex.

#### Reagents
Components and concentration in the test
**R1**: glycine buffer .................. 50 mmol/l
Sulphuric acid
Detergent

**R2**: glycine buffer
Ammonium molybdate .................. 0.4 mmol/l

**Standard (phosphorus)** ................. 5 mg/dl

#### Specimen:
Serum

#### Assay procedure:
Wavelength .................. 340 nm
Optical path .................. 1 cm
Temperature ................. 37°C
Measurement ................. against reagent blank
**substrate start**

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Sample or standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample or standard</td>
<td>-</td>
<td>10 µl</td>
</tr>
<tr>
<td>Dist. water</td>
<td>10 µl</td>
<td>-</td>
</tr>
<tr>
<td>Reagent 1</td>
<td>800 µl</td>
<td>800 µl</td>
</tr>
<tr>
<td>Mix , incubate 5 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>read absorbance A1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>then add</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reagent 2</td>
<td>200 µl</td>
<td>200 µl</td>
</tr>
<tr>
<td>Mix , read absorbance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2 after 5-60 min</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Sample start**

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Sample or standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample or standard</td>
<td>-</td>
<td>10 µl</td>
</tr>
<tr>
<td>Dist. water</td>
<td>10 µl</td>
<td>10 µl</td>
</tr>
<tr>
<td>Monoreagent</td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
<tr>
<td>Mix , incubate 5 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>and read absorbance</td>
<td></td>
<td>against reagent</td>
</tr>
<tr>
<td>against reagent</td>
<td></td>
<td>blank within 60 min</td>
</tr>
</tbody>
</table>

**Reference range**

Serum: 2.6 - 4.5 mg/dl

**3.10.4. Alkaline phosphatase**

**Method**

Kinetic photometric test, according to the international federation of clinical chemistry and laboratory medicine.

**Principle of the test**

\[ \text{P-Nitrophenylphosphate} + \text{H2O} \rightarrow \text{Phosphate} + \text{P-Nitrophenol} \]

**Reagents:**

Components and concentration in the test:
R 1 : 2-Amino-2-methyl-1-propanol  PH10.4................................. 0.90 mol/l
Magnesium acetate.............................1.6 mmol/l
Zinc sulphate .................................. 0.4 mmol/l
EDTA ........................................... 2.0 mmol/l

R 2 : P-Nitrophenylphosphate............ 16.0 mmol/

**Assay procedure**

Wavelength ......................... 405 nm
Optical path.............................. 1 cm
Temperature............................. 37º C
Measurement ......................... against reagent blank

**substrate start .**

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Sample or standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample or standard</td>
<td>-</td>
<td>20 µl</td>
</tr>
<tr>
<td>Dist. water</td>
<td>20 µl</td>
<td>-</td>
</tr>
<tr>
<td>Reagent 1</td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
<tr>
<td>Mix , incubate 1 min</td>
<td></td>
<td>read then add</td>
</tr>
<tr>
<td>Reagent 2</td>
<td>200 µl</td>
<td>200 µl</td>
</tr>
<tr>
<td>Mix , read absorbance after 1min .read absorbance 1,2,3 min</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Sample start**

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Sample or standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample or standard</td>
<td>-</td>
<td>20 µL</td>
</tr>
<tr>
<td>Dist. water</td>
<td>20 µL</td>
<td>-</td>
</tr>
<tr>
<td>Monoreagent</td>
<td>1000 µL</td>
<td>1000 µL</td>
</tr>
<tr>
<td>Mix , read absorbance after 1min .read absorbance 1,2,3 min</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Reference range:**

Adult : U/l
Women 20-50 years 42.98
Men 20-50 years 53-128
Women >60 years          53- 141
Men >60 years               56- 119

3.10.5. Magnesium

Method
Photometric test using xylidyl blue

Principle of the test
Magnesium ions form a purple colored complex with xylidyl blue in alkaline solution. In presence of GEDTA, which complexes calcium ions, the reaction is specific. The intensity of the a purple color is proportional to the magnesium concentration.

Reagents:
Components and concentration in the test:
Reagent:
Ethanolamine  pH 11.0……………………………………… 1mol/l
GEDTA (glycoletherdiamine-tetraacetic acid)…………… 60 µl mol/l
xylidyl blue .......................................................... 110 µl mol/l
Detergents
Standard........................................................................ 2 mg

Specimen: serum.

Assay procedure:
Wavelength................. 520 nm
Optical path ................. 1 cm
Temperature ............... 37
Measurement ............... against reagent blank

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Sample or standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample or standard</td>
<td>-</td>
<td>10 µl</td>
</tr>
<tr>
<td>Dist. water</td>
<td>10 µl</td>
<td>-</td>
</tr>
<tr>
<td>Monoreagent</td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
</tbody>
</table>

Mix, read absorbance against blank after 5-60 at 20-25 C
Reference range:
Women : 1.9-2.5 mg/dl
Men : 1.8-2.6 mg/dl

3.10.6 Sodium

Method
The colorimetric determination of sodium in human serum

Principle of the test
The present method is based on modification of those first describe by Maruna and Triner in which sodium is precipitated as triple salt, sodium magnesium uranyl acetate, the excess uranium then being reacted with ferrocyanide, reducing a chromophore whose absorbance varies inversely as concentration of sodium in the test specimen.

Reagent composition
Filtrate reagent : Uranly acetate 2.1 Mm and magnesium acetate 20 Mm in ethyl alcohol.
Acid reagent : a diluted acetic acid.
Sodium color : potassium ferrocyanide, non reactive stabilizers, and fillers.
Sodium standard : sodium chloride solution : 150 mEq/L of sodium.

Specimen collection
Freshly drawn serum is the specimen of choice and 20 µl amount is required.
Sodium is stable for at least 24 hours at room temperature and 2 weeks when refrigerated.

Procedure
Filtrate preparation
1. Label test tube : blank, standard, control, patient.
2. One ml of filtrate reagent was pipetted to all tubes.
3. fifty µl of samples was added to all tubes and distilled water to all blank.
4. All tubes was shaken vigorously and mixed continuously for 3 minutes.
5. Tubes was centrifuged at high speed for 10 minutes and test the supernatant fluids as described below, taking care not to disturb the protein precipitate.
Color development
1. Test tube was labeled corresponding to above filtrate tubes.
2. One ml of Acid reagent was pipetted to all tubes.
3. Fifty µl of supernatant was added to respective tubes and mix.
4. fifty µl of color reagent was added to all tubes mix.
5. Zero spectrophotometer with distilled water at 550 nm.
6. All tubes was readed and recorded absorbance.

References:
40-155 mEq/L

3.10.7 Estradiol

Principle of the test
The estradiol kit is a solid phase enzyme-linked immunosorbent assay, based on the principle of competitive binding. The microtiter wells are coated with a polyclonal antibody directed towards a unique antigenic site on estradiol molecule. Endogenous Estradiol of patient sample competes with estradiol horseradish peroxidase for binding to the coated antibody. After incubation the unbound conjunction was washed off. The amount of bound peroxidase conjugate was reversely proportional to the concentration of estradiol in the sample. After addition of the substrate solution, the intensity of color developed was proportional to the concentration of estradiol in the patient sample.

Kit component:
1- content of the kit:
A- Microtiterwells,12×8 strips,96 wells. Wells coated with anti-Estradiol polyclonal rabbit antibody.
B- Standard (standard 0-6), 7 vials, 1 ml, the concentration:0, 25, 100, 250, 500, 1000, 2000 pg/ml.
C- Enzyme conjugate, 1 vials, estradiol conjugate to horseradish peroxidase.
E – Substrate solution, 1 vials, 14 ml, use tetramethylbenzidine (TMB ).
D –Stop solution, 1 vials, 14 ml, use contains 0.5M H2SO4.
F –Wash solution, 1 vials, 30 ml

Specimen: Serum.
Specimen collection: The blood was collected by vein puncture, and be allowed to clot, then separated serum by centrifuge at room temperature.

Specimen storage: Specimen was capped and frozen at -20°C prior to assay. Thawed samples were inverted several times prior to testing.

Test procedure

General remarks
- All reagents and specimens were allowed to come to room temperature before use. All reagents were be mixed without foaming.
- Once the test has been started, all steps were completed without interruption.
- New disposal plastic pipette tips were used for each standard, control or sample in order to avoid cross contamination.

Assay procedure

1- Secure the desired number of microtiterwells in the holder.
2- Dispensed 25 µl of each standard, controls and samples with new disposal plastic pipette tips into wells.
3- Dispensed 200 µl enzyme conjugate into each wells.
4- The wells was incubated for 120 min at room temperature without covering the plate.
5- The contents of the wells was shaked briskly.
6- Each well was Added to it 100 µl of substrate solution.
7- The wells was Incubated for 15 min at room temperature.
8- The enzymatic reaction was stopped by adding 50 µl of stop solution to each well.
9- Read the OD at 450±10 nm with microtiter plate reader within 10 min after adding the stop solution.

Expected values:

Males: 10- 36pg/ml

Females :
Pre-menopausal women: 13-191 pg/ml
Post- menopausal women: 11- 65 pg/ml
3.11. Statistical Analysis

All the data obtained from the questionnaire and the results of laboratory investigations were entered in SPSS 11 software and analyzed using z-test in order to examine the proportion of yes if it is significantly smaller or greater than the proportion of No. The t-test was employed in order to detect significant variation among up to two parameters. Additionally, Kolmogorov-Smirnov Test, Sign test and Mann-Whitney non-parametric test were used to test the correlation between the patient group and control group.
Chapter Four

Results
Results

Table 4.1 shows that the mean age for patients group was 53.8±SD years, while the mean age for controls groups was 45.6±SD years. The mean heights for patients groups was 161.2±SD cm, while the mean heights for controls groups was 161.2±SD cm. The mean Weight for patients groups was 71.86±kg, while the mean weights for controls groups was 69.39±kg.

Table 4.1. Demographic characteristics of the study population (patients(n=96 and the control n=96).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>patients</td>
</tr>
<tr>
<td>Age</td>
<td>53.8</td>
</tr>
<tr>
<td>Height</td>
<td>161.2</td>
</tr>
<tr>
<td>Weight</td>
<td>71.7</td>
</tr>
</tbody>
</table>

4.2. Risk factors causes osteoporosis among menopausal women

Table 4.2.1. shows that the women who had last period for more than five years have the highest percentage (36.91%), while the lowest percentage (7.1%) was for women who had last period for ≤ one year.

Table 4.2.1. Distribution of Osteoporotic patients (n=84) according to LMP

<table>
<thead>
<tr>
<th>Time of cessation of last period</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ one year</td>
<td>6</td>
<td>7.1</td>
</tr>
<tr>
<td>1-3 years</td>
<td>19</td>
<td>22.6</td>
</tr>
<tr>
<td>4-5 years</td>
<td>28</td>
<td>33.3</td>
</tr>
<tr>
<td>&gt; five years</td>
<td>31</td>
<td>36.9</td>
</tr>
<tr>
<td>Total</td>
<td>84</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Table 4.2.2. shows that the most common symptoms to the osteoporosis was walking on surface (50%), followed by walking up stairs (46.4%) and then by walking up hills (2.4%). One women (1.2%) was found to have no pain or no symptoms.

**Table 4.2.2. Activities causing joint pain among osteoporotic women n=84**

<table>
<thead>
<tr>
<th>Activities causing joint pain</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walking on surface</td>
<td>42</td>
<td>50.0</td>
</tr>
<tr>
<td>Walking upstairs</td>
<td>39</td>
<td>46.4</td>
</tr>
<tr>
<td>Walking up hills</td>
<td>2</td>
<td>2.4</td>
</tr>
<tr>
<td>No pain</td>
<td>1</td>
<td>1.2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>84</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>

Table 4.2.3. pointed out that the greater the time of practicing physical activity, the less is percentage of osteoporosis among women. The highest percentage of osteoporosis (47.1%) was for women who had the least time of physical activity (≤ 20 min/day).

**Table 4.2.3. Average time of practicing physical activities/min/day by osteoporotic patients n=85.**

<table>
<thead>
<tr>
<th>Average time of practicing physical activities</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 20 min.</td>
<td>40</td>
<td>47.1</td>
</tr>
<tr>
<td>20-40 min.</td>
<td>23</td>
<td>27.1</td>
</tr>
<tr>
<td>40-60 min.</td>
<td>9</td>
<td>10.6</td>
</tr>
<tr>
<td>≥ 60 min.</td>
<td>13</td>
<td>15.3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>85</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>
Load of daily work of osteoporotic women is illustrated in Table 4.2.4. The highest percentage of osteoporosis (43.53%) was for moderate works, while the lowest percentage (5.9%) for light works.

Table 4.2.4 Load of daily work of osteoporotic women (n=85).

<table>
<thead>
<tr>
<th>Load of daily work</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very light</td>
<td>33</td>
<td>38.8</td>
</tr>
<tr>
<td>Light</td>
<td>5</td>
<td>5.9</td>
</tr>
<tr>
<td>Moderate</td>
<td>37</td>
<td>43.5</td>
</tr>
<tr>
<td>Hard</td>
<td>10</td>
<td>11.8</td>
</tr>
</tbody>
</table>

From table 4.2.5. we found that one of the common reasons to stop of working was due to knee, back, and ankle pain (34.4%).

Table 4.2.5. The reason to stop working or walking among osteoporotic women (n=85).

<table>
<thead>
<tr>
<th>The reason to stop working or walking</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory disorder</td>
<td>29</td>
<td>36.5</td>
</tr>
<tr>
<td>Tiredness</td>
<td>31</td>
<td>29.1</td>
</tr>
<tr>
<td>Pain in knee, ankle or back</td>
<td>25</td>
<td>34.4</td>
</tr>
<tr>
<td>Total</td>
<td>85</td>
<td>100.0</td>
</tr>
</tbody>
</table>
4.2.6. Different risk factors leading to osteoporosis in menopausal women (n=96).

<table>
<thead>
<tr>
<th>No</th>
<th>Items</th>
<th>Yes (%)</th>
<th>No (%)</th>
<th>Z-Value</th>
<th>Significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Family history of osteoporosis</td>
<td>70.1</td>
<td>29.9</td>
<td>3.75</td>
<td>&lt; 0.0005**</td>
</tr>
<tr>
<td>2</td>
<td>Calcium treatment intake</td>
<td>21.6</td>
<td>78.4</td>
<td>-5.33</td>
<td>&lt; 0.0005**</td>
</tr>
<tr>
<td>3</td>
<td>Previous fractures</td>
<td>88.5</td>
<td>11.5</td>
<td>7.18</td>
<td>&lt; 0.0005**</td>
</tr>
<tr>
<td>4</td>
<td>Physical activity less than 20 min.</td>
<td>27.0</td>
<td>73.0</td>
<td>4.35</td>
<td>&lt; 0.0005**</td>
</tr>
<tr>
<td>5</td>
<td>Cessation of period for several months</td>
<td>12.3</td>
<td>87.7</td>
<td>-6.78</td>
<td>&lt; 0.0005**</td>
</tr>
<tr>
<td>6</td>
<td>Consume more than one cup of coffee per day and soft drinks</td>
<td>89.5</td>
<td>10.5</td>
<td>7.33</td>
<td>&lt; 0.0005**</td>
</tr>
<tr>
<td>7</td>
<td>Salt intake</td>
<td>92.0</td>
<td>8.0</td>
<td>7.83</td>
<td>&lt; 0.0005**</td>
</tr>
<tr>
<td>8</td>
<td>Having children</td>
<td>98.9</td>
<td>1.1</td>
<td>9.16</td>
<td>&lt; 0.0005**</td>
</tr>
<tr>
<td>9</td>
<td>Sleep disturbances</td>
<td>97.75</td>
<td>2.25</td>
<td>9.01</td>
<td>&lt; 0.0005**</td>
</tr>
<tr>
<td>10</td>
<td>Having uncomfortable chair</td>
<td>96.6</td>
<td>3.4</td>
<td>8.74</td>
<td>&lt; 0.0005**</td>
</tr>
<tr>
<td>11</td>
<td>Emotional status</td>
<td>90.5</td>
<td>9.5</td>
<td>7.42</td>
<td>&lt; 0.0005**</td>
</tr>
<tr>
<td>12</td>
<td>Doses intake of cortisol and thyroxine</td>
<td>71.8</td>
<td>28.2</td>
<td>-4.01</td>
<td>&lt; 0.0005**</td>
</tr>
<tr>
<td>13</td>
<td>Exposure to sunlight</td>
<td>38.4</td>
<td>61.6</td>
<td>-2.15</td>
<td>0.016*</td>
</tr>
<tr>
<td>14</td>
<td>Dairy intake</td>
<td>24.1</td>
<td>75.9</td>
<td>-4.72</td>
<td>&lt; 0.0005**</td>
</tr>
<tr>
<td>15</td>
<td>Cortisol and estrogen intake</td>
<td>31.25</td>
<td>68.75</td>
<td>-3.67</td>
<td>&lt; 0.0005**</td>
</tr>
<tr>
<td>16</td>
<td>Hormone replacement therapy intake</td>
<td>2.41</td>
<td>97.59</td>
<td>-8.67</td>
<td>&lt;0.0005**</td>
</tr>
</tbody>
</table>

** Proportion difference is significant at 0.01 level
* Proportion difference is significant at 0.05 level
The most common risk factors having effect on osteoporosis which were concluded from questionnaire interview are summarized in Table 4.2.6. Women who have relatives suffering from osteoporosis constituted 70.1%. Taking calcium tablets was found to reduced significantly the risk of having osteoporosis (21.6 %v 78.4%, p<0.01). Osteoporosis was also more common among women who had previous fractures (73.0% V 27.0% p<0.01). Consumption of coffee, tea and cola, and large quantities of salt increased osteoporosis (89.5% v 10.5%, p<0.01 and 92.0%v 8.0%, respectively. Other factors that increased osteoporosis included sleeplessness (97.8 v 2.2%, p<0.01). Having uncomfortable chair (96.6% v 3.4%, p<0.01), depression (90.5 % v 9.5%, p<0.01) and taking medication including cortisol and thyroxine (71.8% v 28.2%, p<0.01). In addition factors that reduced osteoporosis including sunlight exposure (38.4% v 61.6%, p<0.01) and hormone replacement therapy (2.4% V 97.6%, p<0.01).

Since the significance for each statement is smaller the $\alpha=0.01$ or $0.05$ level of significance, then we conclude that there is significant difference between the proportions of Yes and No. The sign of the Z-test shows either the proportion of Yes is significantly smaller or greater than the proportion of No. In this case if the sign of the Z-test is positive, then the proportion of Yes is significantly greater than the proportion of No. Otherwise, the proportion of Yes is significantly smaller than the proportion of No.

The sign of the Z-test is positive for statements 1,3,4,5,6,7,8,9,10,11 according to table "4.2.6". We conclude that the proportion of Yes is significantly greater than the proportion of No.

The sign of the Z-test is negative for statements 2,5, 12,13,14,15,16. We conclude that the proportion of Yes is significantly smaller than the proportion of No.
Table 4.2.7. Different risk factors leading to osteoporosis in menopausal women with no recorded osteoporosis (n=96).

<table>
<thead>
<tr>
<th>No</th>
<th>Items</th>
<th>Yes (%)</th>
<th>No (%)</th>
<th>Z-Value</th>
<th>Significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Family history of osteoporosis</td>
<td>12.5%</td>
<td>87.5%</td>
<td>-7.4</td>
<td>&lt;0.0005**</td>
</tr>
<tr>
<td>2</td>
<td>Calcium treatment intake</td>
<td>78.9%</td>
<td>21.0%</td>
<td>5.6</td>
<td>&lt;0.0005**</td>
</tr>
<tr>
<td>3</td>
<td>Previous fractures</td>
<td>14.6%</td>
<td>85.4%</td>
<td>-6.9</td>
<td>&lt;0.0005**</td>
</tr>
<tr>
<td>4</td>
<td>Physical activity less than 20 min.</td>
<td>86.5%</td>
<td>13.5%</td>
<td>7.1</td>
<td>&lt;0.0005**</td>
</tr>
<tr>
<td>5</td>
<td>Cessation of period for several months</td>
<td>9.4%</td>
<td>90.6%</td>
<td>-7.9</td>
<td>&lt;0.0005**</td>
</tr>
<tr>
<td>6</td>
<td>Consume more than one cup of coffee per day and soft drinks</td>
<td>16.7%</td>
<td>83.3%</td>
<td>-6.5</td>
<td>&lt;0.0005**</td>
</tr>
<tr>
<td>7</td>
<td>Salt intake</td>
<td>19.8%</td>
<td>80.2%</td>
<td>-5.9</td>
<td>&lt;0.0005**</td>
</tr>
<tr>
<td>8</td>
<td>Having children</td>
<td>96.9%</td>
<td>3.1%</td>
<td>9.2</td>
<td>&lt;0.0005**</td>
</tr>
<tr>
<td>9</td>
<td>Sleep disturbances</td>
<td>17.8%</td>
<td>82.3%</td>
<td>-6.3</td>
<td>&lt;0.0005**</td>
</tr>
<tr>
<td>10</td>
<td>Having uncomfortable chair</td>
<td>18.8%</td>
<td>81.3%</td>
<td>-6.1</td>
<td>&lt;0.0005**</td>
</tr>
<tr>
<td>11</td>
<td>Emotional status</td>
<td>25.0%</td>
<td>75.0%</td>
<td>-4.9</td>
<td>&lt;0.0005**</td>
</tr>
<tr>
<td>12</td>
<td>Doses intake of cortisol and thyroxine</td>
<td>17.7%</td>
<td>82.3%</td>
<td>-6.3</td>
<td>&lt;0.0005**</td>
</tr>
<tr>
<td>13</td>
<td>Exposure to sunlight</td>
<td>89.6%</td>
<td>10.4%</td>
<td>7.8</td>
<td>&lt;0.0005**</td>
</tr>
<tr>
<td>14</td>
<td>Cortisol and estrogen intake</td>
<td>30.2%</td>
<td>69.8%</td>
<td>-3.9</td>
<td>0.0001**</td>
</tr>
<tr>
<td>15</td>
<td>Dairy intake</td>
<td>82.39%</td>
<td>17.7%</td>
<td>6.3</td>
<td>&lt; 0.0005**</td>
</tr>
<tr>
<td>16</td>
<td>Hormone replacement therapy intake</td>
<td>18.8%</td>
<td>81.3%</td>
<td>-6.1</td>
<td>&lt;0.0005**</td>
</tr>
</tbody>
</table>

** Proportion difference is significant at 0.01 level
* Proportion difference is significant at 0.05 level
The most common risk factors having effect on osteoporosis which were concluded from questionnaire interview are summarized in Table 4.2.7. Women who have relatives suffering from osteoporosis constituted 12.5%. Taking calcium tablets was found to reduced significantly the risk of having osteoporosis 78.9%. Osteoporosis was also more common among women who had previous fractures 14.6%. Consumption of coffee, tea and cola, and large quantities of salt increased osteoporosis (16.77% v 83.3%, p<0.01 and 19.8% v 80.2%, respectively). Other factors that increased osteoporosis included sleeplessness (17.7 v 82.3%, p<0.01). Having uncomfortable chair (18.6% v 81.3%, p<0.01), depression (25.0% v 75.0%, p<0.01) and taking medication including cortisol and thyroxine (17.7% v 82.3%, p<0.01). In addition factors that reduced osteoporosis including sunlight exposure (89.6% V 10.4%, p<0.01) and hormone replacement therapy (30.2% V 69.8%, p<0.01).

Since the significance for each statement is smaller the $\alpha = 0.01$ or 0.05 level of significance, then we conclude that there is significant difference between the proportions of Yes and No. The sign of the Z-test shows either the proportion of Yes is significantly smaller or greater than the proportion of No. In this case if the sign of the Z-test is positive, then the proportion of Yes is significantly greater than the proportion of No. Otherwise, the proportion of Yes is significantly smaller than the proportion of No.

The sign of the Z-test is positive for statements 2,4,8,13,15 according to table "4.2.7.". We conclude that the proportion of Yes is significantly greater than the proportion of No.

The sign of the Z-test is negative for statements 1,3,5,6,7,9,10,11,12,14,16. We conclude that the proportion of Yes is significantly smaller than the proportion of No.
<table>
<thead>
<tr>
<th>No</th>
<th>Item</th>
<th>Healthy (%)</th>
<th>Patients (%)</th>
<th>Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>family</td>
<td>12.5%</td>
<td>70.10%</td>
<td>-57.6%</td>
</tr>
<tr>
<td>2</td>
<td>ca</td>
<td>78.9%</td>
<td>21.60%</td>
<td>57.3%</td>
</tr>
<tr>
<td>3</td>
<td>prev</td>
<td>14.6%</td>
<td>88.50%</td>
<td>-73.9%</td>
</tr>
<tr>
<td>4</td>
<td>phys</td>
<td>86.5%</td>
<td>27.00%</td>
<td>59.4%</td>
</tr>
<tr>
<td>5</td>
<td>cess</td>
<td>9.4%</td>
<td>12.30%</td>
<td>-2.9%</td>
</tr>
<tr>
<td>6</td>
<td>consu</td>
<td>16.7%</td>
<td>89.50%</td>
<td>-72.8%</td>
</tr>
<tr>
<td>7</td>
<td>salt</td>
<td>19.8%</td>
<td>92.00%</td>
<td>-72.2%</td>
</tr>
<tr>
<td>8</td>
<td>children</td>
<td>96.9%</td>
<td>98.90%</td>
<td>-2.0%</td>
</tr>
<tr>
<td>9</td>
<td>sleep</td>
<td>17.7%</td>
<td>97.75%</td>
<td>-80.0%</td>
</tr>
<tr>
<td>10</td>
<td>chair</td>
<td>18.8%</td>
<td>96.60%</td>
<td>-77.9%</td>
</tr>
<tr>
<td>11</td>
<td>emo</td>
<td>25.0%</td>
<td>90.50%</td>
<td>-65.5%</td>
</tr>
<tr>
<td>12</td>
<td>thyrox</td>
<td>17.7%</td>
<td>71.80%</td>
<td>-54.1%</td>
</tr>
<tr>
<td>13</td>
<td>sunlight</td>
<td>89.6%</td>
<td>38.40%</td>
<td>-51.2%</td>
</tr>
<tr>
<td>14</td>
<td>estrogen</td>
<td>30.21%</td>
<td>31.25%</td>
<td>-1.04%</td>
</tr>
<tr>
<td>15</td>
<td>horm</td>
<td>18.75%</td>
<td>2.41%</td>
<td>16.34%</td>
</tr>
</tbody>
</table>

*Table 4.2.8 Difference between proportions of healthy and patients groups*

| Family: Family history of osteoporosis |
| Ca: Calcium treatment intake |
| Prev: Previous fractures |
| Phys: Physical activity less than 20 min. |
| Cess: Cessation of period for several months |
| Consu: Consume more than one cup of coffee per day and soft drinks |
| Salt: Salt intake |
| Children: Having children |
| Sleep: Sleep disturbances |
| Chair: Having uncomfortable chair |
| Emo: Emotional status |
| Thyrox: Doses intake of cortisol and thyroxine |
| Sunlight: Exposure to sunlight |
| Estrogen: Cortisol and estrogen intake |
| Horm: Hormone replacement therapy intake |
We make comparison between the control and patient groups to investigate the difference between proportions of healthy and patients groups for the risk factors leading to osteoporosis among menopausal women which were concluded from questionnaire interview are summarized in Table 4.2.8. we found significant differences for women who have relatives suffering from osteoporosis. Healthy group was significantly smaller than patients group -57.6%. Taking calcium tablets was found in the proportion of healthy group is significantly greater than proportion of patients group 8.1%. Proportion of healthy group is significantly greater than proportion of patients group among women who had previous fractures -73.9%. Consumption of coffee, tea and cola, and large quantities of salt showed significant differences between the control and patient groups (-72.8%, p<0.01 and -72.2%, p<0.01) respectively.

While other factors showed no significant differences between the control and patient groups including: cessation of period for several months (-2.9%, p=0.3), having children (-2.0%, p=0.4) and cortisol and estrogen intake (-1.1%, p=0.4). In addition factors that have significant differences including sunlight exposure (7.2%, p<0.01) and hormone replacement therapy (2.3%, p=0.01).

Table 4.3. Arithmetic mean of variables for patient n=96 and control groups n=96:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients</td>
</tr>
<tr>
<td>Calcium, Normal 8.1-10.6 mg/dl</td>
<td>8.6</td>
</tr>
<tr>
<td>Sodium, Normal 136-146 mmol/L</td>
<td>141.3</td>
</tr>
<tr>
<td>Phosphorus, Normal 2.5-5 mg/dl</td>
<td>5.0</td>
</tr>
<tr>
<td>Magnesium, Normal 1.8-2.3 mg/dl</td>
<td>1.5</td>
</tr>
<tr>
<td>Alkaline phosphatase, Normal 20-140 IU/L</td>
<td>226.9</td>
</tr>
<tr>
<td>Ratio:Ca/P</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Table 4.3. illustrates that the mean of serum calcium, sodium, phosphorus of patients and controls were in the normal range. The mean level of calcium in the patients was 8.6 mg/dl, while the mean calcium level in the controls groups was 9.7 mg/dl. The mean level of Sodium in the patients was 141.3
mmol/L, while the mean of Sodium level in the controls groups was 140.0 mmol/L. The mean of phosphorus level in the patients was 5.0 mg/dl which was little above the normal range, while the mean of phosphorus level in the controls groups was 3.5 mg/dl. The mean of alkaline phosphatase level in the patients was 226.9 IU/L, while the mean of Alkaline phosphatase level in the controls groups was 111.8 IU/L with significant values (p<0.001). The mean of magnesium was below the normal range 1.51 mg/dl, while the mean of magnesium level in the controls groups was 2.21 mg/dl. The mean ratio of calcium / Phosphorus in the patients group was less than 2 (1.7), while the mean ratio of Calcium / Phosphorus level for controls group it was above 2 (3.0).

Table 4.4. Arithmetic mean of estradiol and osteocalcin for patient n=96 and control n=96 groups

Table 4.4 the mean level of estradiol in the patients was below 32 pg/dl 17.3 pg/dl, while the mean of estradiol level in the controls groups was above 32 pg/dl the mean was i.e. 55.2 pg/dl. The mean level of osteocalcin in the patients was 173.3 ng/ml above the normal range, while in control groups it was 67.1 ng/ml.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients</td>
</tr>
<tr>
<td>Estradiol, &quot;Normal 11-66pg/dl&quot;</td>
<td>17.3</td>
</tr>
<tr>
<td>Osteocalcin: &quot;Normal: 20-108ng/ml&quot;</td>
<td>173.3</td>
</tr>
</tbody>
</table>

In order to test whether the mean differences between patients and control groups for magnesium, estradiol, osteocalcin, alkaline phosphatase and ratio = Ca/P are statistically significant, we examine the normality assumption for each variable (Table 4.5).
Table 4.5. Arithmetic mean of magnesium, estradiol, osteocalcin Ca/P and alkaline phosphatase for patient and control groups (Kolmogorov-Smirnov Test)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Significant (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.003**</td>
</tr>
<tr>
<td>Estradiol</td>
<td>0.000**</td>
</tr>
<tr>
<td>Osteocalcin</td>
<td>0.000**</td>
</tr>
<tr>
<td>Ratio = Ca/P</td>
<td>0.845</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>0.001**</td>
</tr>
</tbody>
</table>

** The distribution is not normally distributed at 0.01 significance level

4.3. Results of sign test for magnesium, estradiol and osteocalcin

Sign test is used to test if the mean of magnesium is significantly smaller than 1.8 mg/dl for patients group because the assumption of normality is violated; since the p-value (Sig.) equals 0.003 which is smaller than the level of significance $\alpha = 0.01$.

Table 4.6. Sign test for magnesium

Since the p-value for magnesium equals 4.25E-08 which is smaller than 0.01, then we conclude that the mean of magnesium for patients group differs significantly from 1.8 mg/dl. There is 78% of the patients having magnesium smaller than 1.8 mg/dl, then the mean of magnesium for patients group is significantly smaller than 1.8 mg/dl (Table 4.6).

<table>
<thead>
<tr>
<th>Category</th>
<th>Observed Prop.</th>
<th>Asymp. Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smaller than 1.8 mmg/dl</td>
<td>78%</td>
<td>4.25E-08</td>
</tr>
<tr>
<td>Greater than 1.8 mmg/dl</td>
<td>22%</td>
<td></td>
</tr>
</tbody>
</table>
Since the p-value for estradiol equals 1.58E-014 which is smaller than 0.01, then we conclude that the mean of estradiol for patients group differs significantly from 32 pg/dl. There is 88% of the patients having estradiol smaller than 32 pg/dl, then the mean of estradiol for patients group is significantly smaller than 32 pg/dl (Table 4.7).

Table 4.7: Sign test for estradiol

<table>
<thead>
<tr>
<th>Category</th>
<th>Observed Prop.</th>
<th>Asymp. Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smaller than 32 pg/dl</td>
<td>88%</td>
<td>1.58E-014</td>
</tr>
<tr>
<td>Greater than 32 pg/dl</td>
<td>12%</td>
<td></td>
</tr>
</tbody>
</table>

Since the p-value for osteocalcin equals 2.52E-029 which is smaller than 0.01, then we conclude that the mean of osteocalcin for patients group differs significantly from 108 ng/ml. There is 100% of the patients having osteocalcin greater than 108 ng/ml, then the mean of osteocalcin for patients group is significantly greater than 108 ng/ml (Table 4.8).

Table 4.8: Sign test for osteocalcin

<table>
<thead>
<tr>
<th>Category</th>
<th>Observed Prop.</th>
<th>Asymp. Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smaller than 108 ng/ml</td>
<td>0%</td>
<td>2.52E-029</td>
</tr>
<tr>
<td>Greater than 108 ng/ml</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

4.4. T-test for Ca/P ratio

T-Test is used to test if the mean of Ca/P ratio is significantly smaller than 2 for patients group because the assumption of normality is satisfied; since the p-value (Sig.) equals 0.845 which is greater than the level of significance $\alpha = 0.05$.

Since the p-value for ratio equals 3.67E-021 which is smaller than 0.01, then we conclude that the mean of the ratio for patients group differs significantly.
from 2. Since the sign of the T-test is negative, then we conclude that the mean of Calcium / Phosphorus ratio is significantly smaller than 2.

**Table 4.9 : T-test for Calcium / Phosphorus ratio**

<table>
<thead>
<tr>
<th>Test Value = 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
</tr>
<tr>
<td>-12.203</td>
</tr>
</tbody>
</table>

**4.5. Mann-Whitney U test for magnesium, estradiol and osteocalcin**

Mann-Whitney U test is used to examine the mean differences of magnesium, estradiol, and osteocalcin between patients and control group because the assumption of normality is violated; since the p-values (Sig.) are smaller than the level of significance ($\alpha = 0.01$). Results of Mann-Whitney U test is shown in Table 4.10 and 4.11.

**Table 4. 10 Mean rank by Mann-Whitney test**

<table>
<thead>
<tr>
<th></th>
<th>Magnesium</th>
<th>Estradiol</th>
<th>Osteocalcin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>52.4</td>
<td>52.9</td>
<td>133.5</td>
</tr>
<tr>
<td>Healthy (Control)</td>
<td>135.2</td>
<td>134.5</td>
<td>43.0</td>
</tr>
</tbody>
</table>

As indicated in Table 4.17, the mean rank for magnesium equals 52.4 and 135.1 for patients and controls, respectively, then we conclude that the magnesium mean for patients is significantly smaller than control group. The mean rank for estradiol equals 52.9 and 134.5 for patients and control group, respectively, then we conclude that the estradiol mean for patients is significantly smaller than control group. The mean rank for Osteocalcin equals 133.47 and 43.04 for patients and control group, respectively, that the Osteocalcin mean for patients is significantly greater than control group.
Table 4.11 Comparison of means for magnesium, estradiol, and osteocalcin between patient and control groups

Since the p-value for each variable; magnesium, estradiol, and osteocalcin is smaller than 0.01, then we conclude that significant difference between patients and control group for magnesium, estradiol, and osteocalcin do exist as shown in (Table 4.11).

<table>
<thead>
<tr>
<th></th>
<th>Magnesium</th>
<th>Estradiol</th>
<th>Osteocalcin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mann-Whitney U</td>
<td>372.5</td>
<td>425.5</td>
<td>3.0</td>
</tr>
<tr>
<td>P value (Sig.)</td>
<td>0.000**</td>
<td>0.000**</td>
<td>0.000**</td>
</tr>
</tbody>
</table>

** Mean difference is significant at the 0.01 significance level.

4.6. T-Test is used to examine the mean differences of the Calcium / Phosphorus ratio between patients and controls group.

Independent samples T-Test is used to examine the mean differences of the Calcium / Phosphorus ratio between patients and control group because the assumption of normality is satisfied; since the p-values (Sig.) = 0.845 and 0.243 for patients and control group, respectively which are greater than the level of significance $\alpha = 0.05$.

Table 4.12 : Comparison of means for Calcium / Phosphorus ratio between patient and control groups

<table>
<thead>
<tr>
<th>Levene’s Test for Equality of Variances</th>
<th>t-test for Equality of Means</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>$64$</td>
</tr>
<tr>
<td>Equal variances assumed</td>
<td>97.924</td>
</tr>
<tr>
<td>Equal variances not assumed</td>
<td>-10.965</td>
</tr>
</tbody>
</table>

Since the p-value for Levene’s Test for Equality of Variances equals 0.000 which is smaller than the level of significance $\alpha = 0.05$, then the equal variances between the two groups are not assumed, then the appropriate value of the T-Test equals -10.965 with p-value (Sig.) = 0.000, so we conclude that there is significant difference of mean Ca/P ratio between patients and
control group. Since the sign of T-test is negative then the mean of Ca/P ratio for patients group (1.7) is significantly smaller than the mean ratio for control group (3.0).

4.7. Results of person correlation coefficient

We used Pearson correlation coefficient to examine the direction and the strength of the relationship between each variable and Osteocalcin for patients and control groups (Table 4.13).

There are relationships between osteocalcin and each of weight, and estradiol level. There is a positive relationship between osteocalcin and weight (The Pearson correlation coefficient = 0.185 with P-value = 0.035). There is negative relationship between osteocalcin and estradiol (The Pearson correlation coefficient = -0.284 with P-value = 0.003). The other variables have very weak relationships with osteocalcin as shown in (Table 4.13).

Table 4.13 Relationships between osteocalcin and other variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patients</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pearson Correlation</td>
<td>P-value</td>
</tr>
<tr>
<td>age</td>
<td>-0.028</td>
<td>0.393</td>
</tr>
<tr>
<td>height</td>
<td>-0.062</td>
<td>0.274</td>
</tr>
<tr>
<td>weight</td>
<td>0.185(*)</td>
<td>0.035</td>
</tr>
<tr>
<td>Calcium</td>
<td>-0.116</td>
<td>0.131</td>
</tr>
<tr>
<td>Sodium</td>
<td>-0.044</td>
<td>0.336</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>-0.018</td>
<td>0.431</td>
</tr>
<tr>
<td>Magnesium</td>
<td>-0.153</td>
<td>0.068</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>-0.030</td>
<td>0.384</td>
</tr>
<tr>
<td>Estradiol</td>
<td>-0.284(**)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

* Correlation is significant at the 0.05 level
** Correlation is significant at the 0.01 level

66
Chapter Five

Discussion
Discussion

5.1. Overview

Osteoporosis is a heterogeneous group of abnormal processes characterized biologically by the net loss of bone, which results in a decrease in total mineralized bone without a decrease in the ratio of bone mineral to the organic matrix. Thus there is a decrease in the overall amount of bone (132). Primary osteoporosis is observed mainly in women during early postmenopausal period. Secondary osteoporosis is related to predisposing conditions (133).

Biochemical parameters can give an idea as to the rates of bone formation and resorption. High rate of bone turnover correlates with a low bone mass (134). In general, women lose about 1% of their bone density per year during and after menopause. However, nearly 35% of women lose bone at a faster rate during the late Perimenopause period. Biochemical markers can detect women who are considered “rapid losers” that is, those who lose 3% to 5% of bone per year (135). In recent years, a panel of new, simple to use kits for determining bone markers became available for the diagnosis, treatment and monitoring of osteoporosis and other metabolic diseases of bone tissue (136).

The aim of this study is to make women aware of the consequences of osteoporosis and focus on the risk factors that cause the osteoporosis. The introduction of new laboratory investigations like osteocalcin can help physicians for prefect diagnosis, influence treatment decisions, and ultimately reduce fracture outcome.

In the current study, we have compared between two groups of women; the first group represented by postmenopausal women without osteoporosis (controls) and the second group included postmenopausal women with osteoporosis (patients).
5.2. Serum calcium

Total serum calcium was normal in both postmenopausal women with osteoporosis and postmenopausal women without osteoporosis because osteoporosis causes decrease in total mineralized bone without a decrease in the ratio of bone mineral to the organic matrix. Thus there is a decrease in the overall amount of bone. The mean of serum calcium in the patients group was 8.62 mg/dl, while the mean of serum calcium in the controls group was 9.68 mg/dl and the normal range for serum calcium is (8.1-10.6 mg/dl) so both groups were within normal range. Like our findings, several studies showed that serum calcium determination was of no significant values for diagnosis of osteoporosis as their results were within normal range (129, 131, 137, 138, 139).

5.3. Serum phosphorus

Total serum phosphorus was normal in both postmenopausal women with osteoporosis and postmenopausal women without osteoporosis because osteoporosis causes decrease in total mineralized bone without a decrease in the ratio of bone mineral to the organic matrix. Thus there is a decrease in the overall amount of bone. The mean of serum phosphorus in the patients group was 2.04 mg/dl, while the mean of serum phosphorus in the controls group was 3.54 mg/dl and the normal range for serum phosphorus is (2.2-5 mmol/l) so both groups were within normal range. Like our findings, several studies showed that serum phosphorus determination was of no significant values for diagnosis of osteoporosis as their results were within normal range (129, 131, 137, 138, 139).

5.4. Serum sodium

Serum sodium was slightly elevated in postmenopausal women with osteoporosis while it was normal in postmenopausal women without osteoporosis. The mean level of serum sodium in the patients group was 148 mEq/L, while in the controls group it was 140.02 mEq/L, the normal range for serum sodium is (136-146 mEq/L).
A study examining bone mass and Na intake showed that changes in urinary Na were significantly and negatively correlated (P<0.05) with changes in BMD of the hip and ankle in postmenopausal women (141). Other studies were not in agreement with our study. It was reported that, in post-menopausal women there was no evidence that salt intake and BMD are inversely related (142). The assessment of Na intake was inadequate, being based on a single 24 h dietary recall at baseline; thus, the results of this study might unreliable.

5.5. Serum magnesium

Serum magnesium was below the normal range in postmenopausal women with osteoporosis and within normal range in postmenopausal women without osteoporosis. The mean of serum magnesium in the patients group was 1.51 mg/dl, while in the controls group it was 2.21 mg/dl, the normal range for serum magnesium is (1.8 – 2.3 mg/dl).

Similar study reported that the mean of serum magnesium in the patients group (postmenopausal women) was 11% of patients group was less than 1.9 mg/dl, and this is significant value. And this result helps in diagnosis of the osteoporosis (143).

Similar study reported that the mean of serum magnesium in the patients group (postmenopausal women) was 11% of patients group was less than 1.9 mg/dl, and this is significant value. And this result helps in diagnosis of the osteoporosis (144).

The mean level of serum magnesium is significantly smaller than 1.8 mg/dl for patients group (p=0.003). About 78% of the patients have magnesium levels below 1.8 mg/dl. This phenomenon requires more investigation e.g relationship of Mg to the level of parathyroid hormone and kidney function tests of the target group. As Mg is abundant intracellularly there may be defect in the transport of Mg in the favour of more intracellular levels.
5.6. Serum alkaline phosphatase

Total serum alkaline phosphatase in postmenopausal women with osteoporosis was higher than normal range while the postmenopausal women without osteoporosis was within normal range. The mean of serum alkaline phosphatase in the patients group was 226.85 IU/l, while in the controls group it was 111.77 IU/l, normal range for serum alkaline phosphatase is (20–40 IU/l).

Like our findings, several studies showed that the serum alkaline phosphatase level was significantly higher in postmenopausal women with osteoporosis than in postmenopausal women without osteoporosis (144, 139, 136).

There was significant relationship (p<0.001) between serum alkaline phosphatase and osteoporosis. As this enzyme function in the metabolism of bone tissues, it may be expected that its increased activity is a way to compensate for lost Ca/P by recruiting more of these elements to build new bone tissue.

5.7. Serum estradiol

Total estradiol was normal in both postmenopausal women with osteoporosis and postmenopausal women without osteoporosis. The mean of serum estradiol in the patients group was 17.30 pg/dl, while in the controls group it was 55.23 pg/dl and the normal range for serum estradiol is 11–66 pg/dl. So, both groups are within normal range. However according to our method of assay we noticed that the mean of serum estradiol in the patients group was below 32 pg/dl while the mean of serum estradiol in the controls group was above 32 pg/dl.

Several studies showed that women with detectable estradiol levels (5–32 pg/mL) had about 6–7% higher BMD than women with undetectable levels (<5 pg/mL). Women in the first group had less bone loss at the hip than women with undetectable levels. Women with a minimum of 32 pg/ml estradiol averaged only 0.1% annual hip bone loss, while women with levels below 5 pg/mL averaged 0.8% annual hip bone loss (145,146,147).
Our results showed the mean of estradiol is smaller than 32 pg/dl for patients group \((p= 0.000)\). We noticed that 88% of the patients having estradiol below 32pg/dl. Although the normal range of estradiol is broad (11-66 pg/dl), it is apparent that there is deficiency in this hormone in the patient group which adds to other factors leading to osteoporosis.

5.8. Serum osteocalcin

The normal range of osteocalcin is \((20-108 \text{ ng/ml})\). The mean of osteocalcin level in the patients \((173.3 \text{ ng/ml})\) was above the normal range and, while in the controls groups it was 67.12 ng/ml. The p-value was \(p= 0.000\). There were 100% of the patients having osteocalcin greater than 108ng/ml, then the mean of osteocalcin for patients is greater than 108 ng/ml.

It was reported that the mean of serum osteocalcin in osteoporosis subjects \((26.70 \text{ ng/mL})\) was higher than normal \((19.16 \text{ ng/mL})\) subjects \((p<0.005)\). The incidence of osteoporosis was related to the increase of age. Serum osteocalcin level was related to the severity of diagnosis \((148)\).

A recent study reported that mean value of osteocalcin was significantly higher in postmenopausal osteoporotic than in nonosteoporotic women \((P < 0.05)\). The mean value for postmenopausal osteoporotic was 10.31 ng/ml while the mean value for postmenopausal nonosteoporotic women was 7.7.5 ng/ml \((130)\)

A since osteocalcin levels are elevated in patients, while estradiol levels are low, this finding is of value in determining if the patient is likely to suffer from osteoporosis in the near future. So we recommend to do both tests for women who are at risk for the purpose of warning women to take care and adopt the appropriate precautions.

5.9. Serum ratio of calcium / Phosphorus

The mean ratio of calcium / Phosphorus in the patients group was less than 2 \((1.73)\), while for controls group it was greater than 3.03. This results is expected as both ions are key players in bone metabolism. Despite the finding
that both calcium and phosphorus levels were in the normal range, it seems that ca/p ratio is more predictive than each one alone.

5.10. Risk factors from questionnaire for patient group:

Women who had last period for more than five years had the highest incidence of osteoporosis. The most common activity as predisposing factor of osteoporosis was walking on surface and walking up stairs. The highest percentage of osteoporosis was for women who had physical activity<20 min. day and also for women had moderate work. One of the common reasons for stop of working was knee pain, back, and ankle pain while the lowest percentage stands for tiredness.

The most common risk factors of osteoporosis include women having relative who is suffering from osteoporosis, person had fractures in the past or not, exercises have also effect on the osteoporosis and the percentage for saying No was 73.00 %, drinking coffee, tea, cola every day, and having large amount of salt in food. Other factors that affect the osteoporosis occurrence included sleeplessness, having difficulty in standing from sitting on chair, emotional state of women, taking medication including cortisol and estrogen, sitting in the sunlight and the kind of food e.g cheese, or milk.

Similar study reported that a women who had physical activity less than 20 min and suffered osteoporosis was 6.8%, and osteoporotic women who had exposure to sunlight was 12.3% (149).

Similar study reported that osteoporotic women who used glucocorticoids was 9%, and women who had history of low energy fractures 1.5% (150).

In comparison with other studies our questionnaire findings emphasize the point that each community has its own characteristics in relation to social life, dietary habits as well as practicing exercise. However, factors related to each individual e.g administration of different drugs may affect the outcome of bone metabolism. All the above mentioned factors need more investigations.
Chapter Six

Conclusions and Recommendations
Conclusions

- Assay of osteocalcin seems to be helpful for diagnosing and screening of osteoporosis.
- The present study provides strong evidence that estradiol level is a useful tool for the diagnosis of osteoporosis in menopausal women as it shows low levels in comparison with controls.
- Alkaline phosphatase was higher in postmenopausal women with osteoporosis than normal range subjects.
- The magnesium serum level was below the normal range in postmenopausal women with osteoporosis; about 78% of the patients have magnesium levels below 1.8 mg/dl.
- The calcium and phosphorus were within normal range in postmenopausal women with osteoporosis.
- Serum sodium level was slightly elevated in postmenopausal women with osteoporosis. This may contribute to osteoporosis and increased risk of fracture.
- Calcium/phosphorus ratio in patients group was lower in comparison to control group.
- It appears that group of risk factors e.g obesity, unhealthy dietary habits, unexposure to sunlight contribute to occurrence of osteoporosis.
**Recommendation**

- Introduction of new laboratory investigations like osteocalcin and estradiol to help physicians in prefect diagnosis and influence treatment decisions, and ultimately reduce fracture outcome.
- Good calcium and vitamin D supplementation to prevents osteoporotic fractures.
- Limitation consumption of cola-flavored soft drinks.
- Decrease caffeine intake to fewer than 4 cups of coffee per day.
- Decrease salt intake in food per day.
- Exercise 3 times per week, for at least 20–30 minutes each time, is recommended.
- Exposure to sunlight 5 - 6 times a week.
- Thin, underweight, or small-boned women all have a greater chance of developing osteoporosis.
- Recommend screening postmenopausal women to prevent fragility fractures to identifying postmenopausal women with low bone mineral density and that treating osteoporosis can reduce the risk of fractures in this population.
- Family history play great effect on having osteoporosis so any women have maternal or paternal with osteoporosis should make screening test to prevent the osteoporosis.
- More research is needed to cover various aspects of osteoporosis.
- It is recommended to establish a professional society in Palestine that deal with osteoporosis as a public health problem.
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Appendices
Appendix A


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<th>سؤال</th>
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<tbody>
<tr>
<td>1</td>
<td>هل لك قريب يعانى من هشاشة عظام؟</td>
</tr>
<tr>
<td>2</td>
<td>هل تأخذين كمية مناسبة من كالسيوم؟</td>
</tr>
<tr>
<td>3</td>
<td>هل تأخذين أمراض كالسيوم؟</td>
</tr>
<tr>
<td>3</td>
<td>هل عانتين من أى كسر في ماضي؟</td>
</tr>
<tr>
<td>4</td>
<td>كام عمرك عند انقطاع الحيض؟</td>
</tr>
<tr>
<td>5</td>
<td>أمارس الرياضة كل يوم؟</td>
</tr>
<tr>
<td>6</td>
<td>توقيف الحيض لمدة شهر (استثناء حمل وارضاع)</td>
</tr>
<tr>
<td>7</td>
<td>أشرب أكثر من أثنتين كوب من القهوة أو مشروب غازي كل يوم</td>
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<td>8</td>
<td>أتناول الكثير من الملح</td>
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<tr>
<td>9</td>
<td>هل لديك أولاد؟</td>
</tr>
<tr>
<td>10</td>
<td>ها تعاني من اضطرابات في اليوم؟</td>
</tr>
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<td>11</td>
<td>هل تجد صعوبة في نهوض من على كرسي؟</td>
</tr>
<tr>
<td>12</td>
<td>أني أميل إلى النقل كثيرا، أنا أكون غير سعيدة أكثر من أكون سعيدة</td>
</tr>
<tr>
<td>13</td>
<td>هل تأخذين جرعات عالية من أدوية عدة درقية أو من الكورتيزون أو أدوية لمدة طويلة مثل الرئو أو سكري؟</td>
</tr>
<tr>
<td>14</td>
<td>أ تعرض لشمس أكثر من ثلاثة مرات في اليوم؟</td>
</tr>
</tbody>
</table>
الجزء الثاني: (بخصوص فترات الحيض)

1. متي كانت آخر فتره حيض لديك؟
   - أقل من سنة
   - 1-3 سنوات
   - 4-5 سنوات
   - أكثر من 5 سنوات

أسئلة عن الرياضة:

1. هل تشعرين بالملام في المفاصل في أحد لأوضاع لآتية؟
   - المشي على أرض مسطحة
   - صعوددرج
   - صعود مكان مرتفع
   - لا يوجد

2. كم متوسط فترة عملك اليومي؟
   - 60 دقيقة أو أكثر
   - 40-60 دقيقة
   - 20-40 دقيقة
   - 20 دقيقة أو أقل

3. كيف تصفين عملك اليومي؟
   - خفيف جداً
   - خفيف
   - متوسط
   - صعب

4. أثناء العمل، إذا توقفت للراحة: 
   - نقص في النفس
   - تعب
   - ألم في الركبة، الكاحل، الظهر

أسئلة عن الغذاء:

1. أشرب الحليب ( )
2. آكل زيادي ( )
3. كل الجبن ( )
## Appendix B

**Questionnaire**

Name of patient: ____________  
Age: ____________  
Height: ____________  
Weight: ____________ 

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<thead>
<tr>
<th>No</th>
<th>Statement</th>
<th>Yes (%)</th>
<th>No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Family history of osteoporosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Calcium treatment intake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Previous fractures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Physical activity less than 20 min.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Cessation of period for several months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Consume more than one cup of coffee per day and soft drinks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Salt intake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Having children</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Sleep disturbances</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Having uncomfortable chair</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Emotional status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Doses intake of cortisol or thyroxine</td>
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<td></td>
</tr>
<tr>
<td>13</td>
<td>Exposure to sunlight</td>
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<td>14</td>
<td>Dairy intake</td>
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<td>15</td>
<td>Cortisol and Estrogen intake</td>
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<td>16</td>
<td>Hormone replacement therapy intake</td>
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1- Distribution of Osteoporotic patients according to last period:

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<th>Time of cessation of last period</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
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<td>&lt; one year</td>
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</tr>
<tr>
<td>1-3 years</td>
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</tr>
<tr>
<td>4-5 years</td>
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<tr>
<td>&gt; five years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
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2- Activities causing joint pain

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Percent</th>
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</thead>
<tbody>
<tr>
<td>Walking on surface</td>
<td></td>
</tr>
<tr>
<td>Walking upstairs</td>
<td></td>
</tr>
<tr>
<td>Walking up hills</td>
<td></td>
</tr>
<tr>
<td>No pain</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
</tr>
</tbody>
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3- Load of daily work

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very light</td>
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</tr>
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<td>Light</td>
<td></td>
</tr>
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<td>Moderate</td>
<td></td>
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<tr>
<td>Hard</td>
<td></td>
</tr>
<tr>
<td>Total</td>
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</tr>
</tbody>
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### 4. Average time of practicing physical activities

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 min. or more</td>
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</tr>
<tr>
<td>40-60 min.</td>
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</tr>
<tr>
<td>20-40 min.</td>
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<td>20 min. or less</td>
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<td><strong>Total</strong></td>
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### 5. The reason to stop working or walking:

<table>
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<tr>
<th>Reason</th>
<th>Frequency</th>
<th>Percent</th>
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</thead>
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<td>Pain in knee, ankle or back</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

93
Appendix C

الموضوع / تسهيل مهمة البحث

نود أن نعلم سيداتكم بالطالبة / هدي هنينية ظالمة في ماجستير العلوم الحياة -
تخصص تحليل طبية في الجامعة الإسلامية تقوم بإجراء بحث بعنوان:

Occurrence of Osteoporosis Among Menopausal Woman in Gaza Strip

والطالبة تحتاج للحصول على العينات والمعلومات حتى تتمكن من إجراء البحث.

لذا نرجو من سيداتكم تسهيل مهمة الباحثة.

وكلكم جزيل الشكر والتقدير

مدير برنامج ماجستير العلوم الحياة
د. عيوب باسر النشاشي

Islamic University P. Bex 108 ALRimal Gaza Palestine
Tel: (970/8) 2860700 Fax: (970/8) 2860700 2863552 e-mail: public@mail.lugaza.edu Web Site: www.lugaza.edu
Appendix D

The Islamic University of Gaza

(Name)

The occurrence of Osteoporosis among Menopausal Woman in Gaza Strip

(Name)

Director of the Program in Advanced Studies

Abd al-Husayn al-Qushari