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RESEARCH ARTICLE

THE ASSOCIATION BETWEEN ESTROGEN STATUS AND OTHER BIOCHEMICAL MARKERS OF BONE TURNOVER INCLUDING SERUM GALACTOSYL HYDROXYLYSINE IN OSTEOPOROSIS AND OSTEOPENIA

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ABSTRACT

Osteoporosis is a chronic worldwide problem. It is a systemic skeletal disease characterized by low bone mass and structural deterioration of bone tissue leading to bone fragility. Cessation of ovarian function is the most widespread cause for osteoporosis in postmenopausal women. This study included 37womenaged 40 to 60 years. They were categorized into 3 groups according to their bone mineral density (BMD): Group I: 15 Normal control (T-score up to -1.5), Group II:12 Osteopenic women (T-score between– 1.5 to –2.5) and Group III:10 Osteoporotic women (T-score below– 2.5). For all subjects, dual energy X-ray absorptiometry (DEXA) was performed. Osteocalcin (OC),alkaline phosphatase (ALP), free galactosyl hydroxylysine (Gal-Hyl),calcium (Ca),inorganic phosphorus (P) and estradiol (E_2)were measured in serum, whereas, deoxypyridinoline (Dpd) and creatinine levels in urine. Simultaneously both osteopenic and osteoporotic groups showed significant decreases in BMD when compared to the controls. Osteocalcin, ALP and Gal-Hyl showed significant increase ($p<0.0001$) among the osteopenic and osteoporotic groups versus the control group. Significant decrease in E_2 levels were obvious among the osteopenic ($p<0.0001$) and osteoporotic ($p<0.0001$) women when judged against the controls. Urinary Dpd was significantly increased in the second and the third group ($p<0.001$) together. In osteoporotic group, significant negative correlations were observed between OC and BMD. Positive correlations were detected among the osteoporotic group between OC and ALP and between OC and Gal-Hyl. High significant negative correlations were confirmed between E_2 and OC among both the osteopenic and the osteoporotic groups. Also, a significant negative correlation was established between E2 and Dpd in the osteoporotic group. In comparing between osteopenic and osteoporotic groups, significant decrease was recognized in BMD and significant increase was predicted regarding ALP, ($p<0.05$) Gal-Hyl ($p<0.0001$) and Dpd ($p< 0.001$). In conclusion, the decreasing level of E_2 in early and post-menopausal women is the main cause of osteoporosis. Also, the measurement of serum Gal-Hyl may be of clinical value to identify groups at higher risk of osteoporosis and to predict bone loss.

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INTRODUCTION

Bone is formed of a dense matrix, in which spider cells called "osteocytes" are embedded (Johnell and Kanis, 2006). Ninety percent of the bone is extra-cellular matrix and 10% is water. Inorganic minerals form 60- 70% of bone matrix (calcium phosphates with traces of sodium, magnesium, fluoride, iron and other ions). The organic component of the matrix is type I collagen (90%) with non-collagenous proteins (Gullberg *et al.,* 1997). At the beginning of the fourth decade, a small deficit in bone formation occurs with every resorption and formation cycle. Accordingly, age related bone loss is a normal and predictable biological phenomenon (Rogmark *et al.,* 1992).

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Eriksen and Langdal, (Jaglal *et al.,* 2005) identified osteoporosis as a worldwide problem. It is regarded as a disorder characterized by decrease bone mass per unit of bone volume without abnormality in either the composition or proportion with deterioration of bone micro-architecture with a consequent increase in bone fragility. As osteoporosis is a condition of imbalance between bone resorption and bone formation (Kannus *et al.,* 2006) and since the rate of bone loss varies significantly from one individual to another resulting in different degrees of osteoporosis so, assessment of bone metabolism by the specific biochemical markers is helpful to select osteoporotic patients with high or low turnover (Nymark *et al.,* 2006). Klosla *et al.* (2009) reported that bone loss in women is accelerated during peri-menopausal and postmenopausal periods and is caused by gonadal

insufficiency. Estrogen that has a key role in normal physiology of the skeleton, maintains bone mass as long as the production is sufficient. It has been shown that serum bioavailable estrogen is independent predictor for bone density in post menopause. Increased bone turnover at menopause is driven by bone resorption after osteoclasts are recruited from bone marrow, which is caused by dropping of estrogen levels (Johansson *et al.,* 2004). Bone mineral density measured by densitometry is the elective parameter for the diagnosis of osteopenia and osteoporosis. Dual energy X-ray absorptiometry is the diagnostic measure of choice for osteoporosis. It provides measurement of the spine, femur and total body minerals. This method provides good diagnostic sensitivity for overall fracture risk. Because DEXA has a relatively small precision error, it allows much more sensitive and rapid detection of bone changes (*AAFP*). Usually the estimated bone density is given as a Z-score or a T-score which is the difference between the actual bone mass and the mean peak bone mass, and is also expressed as a fraction of SD (Cummings *et al.,* 1995). Several studies indicated that screening for bone markers might be useful for improving the assessment of osteoporotic women in combination with bone mass measurement (Johnell *et al.,* 2004). Biochemical markers of bone turnover reflect either the rate of bone formation as OC and ALP or the rate of bone resorption. The most sensitive and specific markers of bone resorption have been based on the measurement of urinary analytes, such as Gal-Hyl and Dpd (Leslie *et al.,* 2009). Serum-based measurements are likely to provide better clinical utility because the within subject variability of serum markers may be better. In addition, serum based measurements of bone resorption could potentially allow direct comparison with markers of bone formation, all of which are measured in serum (Cummings *et al.,* 1995).

Segrest and Cunningham, (Lydick *et al.,* 1998) observed that hydroxy-lysine residues in collagen are less abundant than hydroxy-proline, and are not reused in collagen biosynthesis. A variable proportion of hydroxy-lysine residues are galactosylated to form Gal-Hyl, and this particular form is abundant in bone type I collagen (Cadarette *et al.,* 2000). The collagen cross-links pyridinoline and deoxy- pyridinoline (Dpd) are formed during maturation of extra-cellular collagen fibrils and measurement of these components in urine have been shown to provide valid clinical markers of collagen degradation (Cadarette *et al.,* 2001). The enzyme ALP has been used as an indicator of osteoblastic activity. It is involved in making phosphate available for calcification of bone and some enzyme leaks into the serum where it can be measured (Cadarette *et al.,* 2000). *Weaver,* (Mauck *et al.,* 2005) demonstrated that osteoblastic activity is associated with serum OC, one of the proteins found in relatively high concentration in bone.

The human OC molecule contains 49 amino acids and has a molecular weight of 5-8 Kda (Kanis *et al.,* 2008). As OC is synthesized by osteoblasts in bone and only small amounts in dentin, therefore, serum OC originates nearly exclusively from bone and its measurement provides a marker of osteoblast activity (Bettica *et al.,* 1996). The present study was designed to find out the most sensitive serum and urinary markers of osteoporosis among Saudi women and to clarify the relationship between E_2 deficiency and these markers in peri-menopause, early or postmenopausal women without hormonal replacement therapy.

SUBJECTS AND METHODS

This study was carried out on 37 women aged between 40 and 60 years. They were selected to match as much as possible the same age, marital, educational and socioeconomic status. All individuals were subjected to the following:

A) Clinical evaluation

I. Preliminary consent was obtained from all participants.
II. History and general examination:
- Personal history: age, duration of menopause and special habits (smoking and physical exercise).
- Complaint: bone-ache and backaches if present.
- Past history: history of previous operation as thyroidectomy.
- Weight and height were measured while the individual was bare-footed.

III. Exclusion criteria:
- Liver, kidney, endocrinal or rheumatological diseases.
- Drugs affecting bone and calcium metabolism.
- Fractures within 6 months before the time of study.
- Immobilization.

B) Investigations

Assessment of BMD was done for every subject using DEXA (McLaren *et al.,* 1992). The usual regions measured are the lumbar spine and femur. The percentage and T-score of BMD were estimated in antero-posterior (AP) spine and left (Lt) femur. The studied subjects were categorized into the following three groups according to their BMD (WHO) into:

Group I: 15 Normal women (control): T-score up to (-1.5).
GroupII: 12 Osteopenic women: T-score between (-1.5to– 2.5).
Group III: 10 Osteoporotic women: T-score below (-2.5).

Biochemical studies

Blood samples

Fasting blood samples were obtained from each women after overnight fasting from the anticubital vein, serum was separated and stored in clean plastic tubes after being divided into aliquots and frozen at 20^{o}C until assayed.

- Serum OC by enzyme–linked immunosorbent assay (ELISA) (Seibel *et al.,* 1993).
- Serum ALP activity by colorimetric determination as it is the most sensitive marker for bone formation (Christiansen *et al.,* 1987).
- Serum Ca by UNICAM-939 atomic absorption spectrometer (Product of England) according to Brown, (Hansen *et al.,* 1991).
- Serum inorganic phosphorus by direct colorimetric method with molybdenum blue (Raisz, 2005).
- Serum E2 using ACTIVETM E2 DSL-4300 RIA kit. Product of Diagnostic System Laboratories, Inc., USA (Nayak *et al.,* 2006).
- Determination of Gal-Hyl by HPLC (Díez-Pérez *et al.,* 2003)

A) Preparation of sample and drerivatization

- Preliminary ultra filtration of serum (Centrifree™ MPS-1 micro partition system with YMT membranes.
- A 500-µL aliquot of each serum sample was centrifuged at 700*g* for 90 min.
- 100 µL of the ultra filtrate were pipetted into 1.7-mL polypropylene tubes.
- 50 µL of sodium carbonate solution and 50 µLof dansyl chloride solution were added.
- The mixture was vortex-mixed briefly.
- The samples were incubated in a water bath at 60°C for 30 min.
- The samples were cooled in a refrigerator at 4 °C for 15 min.
- 100 µL were injected onto the HPLC column.

B) HPLC conditions for quantitation of Gal- Hyl

- L-Lysine was purchased from Sigma (Sigma chemical COPO Box 1450 and st. Lous, Mo 63178 USA).
- HPLC equipment included a GBC instrument Model LC1150 dual head pump, Australia, Serial No., 4452602777.
- The instrument is supplied with auto-sampler LC 1610, online degasser model Gt 104.
- We used C_{18} reversed-phase column (4.6x 70 mm, 3 μm particle size).
- Mobile phase:

Buffer A: 125ml/L acetonitrile and 50 ml/ L isopropanol made up to volume with sodium acetate solution, pH 6.3.

Buffer B: 500 ml/L acetonitrile and 10 ml/L isopropanol made up to volume with sodium acetate solution, pH 6.5.

- All reagents were HPLC- grade and filtered through 0.45 μm filters before use.
- The flow rate was 1.0 ml/min. The ratio of mobile phase buffers A and B varied from 9:1 (A: B) at the time of injection to 1:9 at 50 min.
- Perkin Elmer Luminescence Spectrometer Detector was used (excitation and emission wavelengths were 366 and 490 nm respectively.
- Retention time (T_R) was 36 min.

*NB.*The attenuation factor of detector was adjusted to the lowest possible value to increase sensitivity and at the same time guard against background noises.

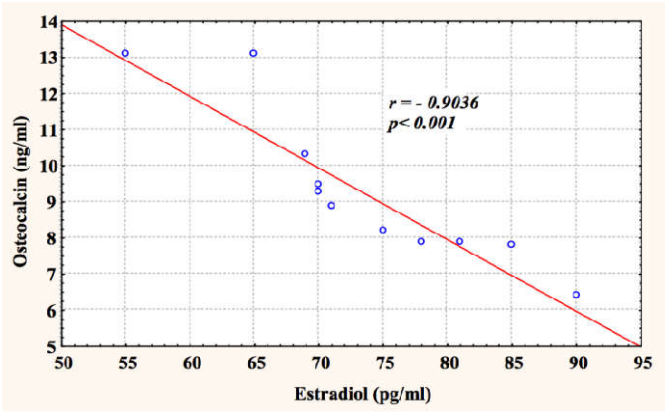

Fig. 1. Correlation between E2 and OC in osteopenic patients

- Quantification was calculated by peak area, using an external calibrator didansylated L-lysine.
- All data were positively skewed and were logarithmically transformedbefore statistical analysis.

Freshly voided first morning midstream urine specimens were collected for estimation of:

- Deoxypyridinoline (Dpd) using Radioimmunoassay (Varney *et al.,* 1999).
- Creatinine using kinetic tests without deproteinization (Marín *et al.,* 2004).

Statistical analysis

The calculation, statistical analysis and graphics were carried out by means of micro net Pentium IV personal computer, IBM compatible. The statistical calculations were done using SPSS (version 15).

RESULTS

The results were demonstrated through the following tables and figures:

Table (1) demonstrates a comparison between some laboratory data for the osteopenic groups and the controls. It is evident that there is no significant difference between both groups as regards the age. While significant decreases were observed as regards bone mineral density including AP Spine BD%, AP Spine BD T score, Lt Femur BD % and Lt Femur T score. Both OC, ALP and Gal- Hyl were significantly higher among the osteopenic women when compared to the control group. Furthermore, urinary Dpd was obviously increased among the osteopenic than the controls. No significant difference between the examined two groups was noticed in Ca levels while P levels showed slight decrease among group II when compared to the control. But for the E_2 value, a significant lower level was observed among the diseased group. Table (2): clarifies a comparison between some laboratory data for the osteoporotic group and the controls. It is evident that there is a significant difference between both groups as regards the age. While significant decreases were observed as regards bone mineral density including AP Spine BD %, AP Spine BD T score, Lt Femur BD % and Lt Femur T score. Both OC, ALP, Gal-Hyl were significantly higher among the osteoporotic women when compared to the control group.

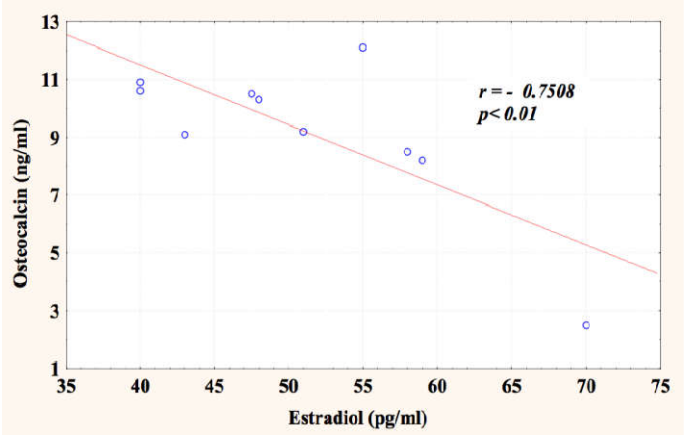

Fig. 2. Correlation between E2 and OC in osteoporotic patients

Table 1. Comparison between osteopenic group and controls regarding some investigated parameters

Sub-Group / Variable	Normal (n= 15)			Osteopenic (n= 12)			*P value*
	Mean	± SE	Range	Mean	±SE	Range	
Age	52.27	1.4	40- 60	48.9	1.9	40 – 60	>0.05
Ap Spine BD %	101.2	2.3	86- 119	82.9	0.96	77 – 88	*<0.05*
Ap Spine BD T score	-0.09	0.23	-1.4 – 1.9	-1.8	0.1	-2.3 -1.5	*<0.001*
Lt Femur BD %	109.33	2.7	95 – 138	94.9	2.6	73- 110	*<0.001*
Lt Femur T score	.82	0.22	-0.4 – 3.2	-0.29	0.22	-2.2 – 0.8	*<0.05*
OC (ng/ml)	5.23	0.61	1.8 – 9.3	9.28	0.59	6.4 – 13.1	*<0.0001*
ALP (U/L)	209.5	6.1	178 – 255	275	5.8	257 – 331	*<0.0001*
Gal-Hyl (nmol/L)	61.18	1.2	53.4 - 70	80.25	0.95	74.1– 86.8	*<0.0001*
Calcium (mg/dl)	8.49	0.34	6.9 – 11.3	8.33	0.33	6.9 – 11.1	>0.05
Phosphorus (mg/dl)	3.45	0.19	2.2 – 4.9	3.0	0.81	2.3 – 3.5	>0.05
Dpd (nM/mM creat.)	6.27	0.17	5.5 – 8.4	7.9	0.19	7.1- 9.4	*<0.0001*
Estradiol (pg/ml)	109.31	6	68 – 160	73.3	2.7	55 – 90	*<0.0001*

Non significant= P > 0.05; Significant = P < 0.05; Highly significant = P < 0.001; Very highly significant = P < 0.0001

Table 2. Comparison between osteoporotic group and controls regarding some investigated parameters

Sub-Group / Variable	Normal (n= 15)			Osteoporosis (n= 10)			*P value*
	Mean	± SE	Range	Mean	± SE	Range	
Age	52.27	1.4	40- 60	57.9	0.66	55 – 60	*<0.05*
Ap Spine BD %	101.2	2.3	86- 119	75.8	1.2	72 – 86	*0.0001*
Ap Spine BD T score	-0.09	0.23	-1.4 – 1.9	-2.67	0.1	-3.2 - -2.5	*<0.0001*
Lt Femur BD %	109.33	2.7	95 – 138	88.2	5.1	70 – 127	*<0.001*
Lt Femur T score	.82	0.22	-0.4 – 3.2	-1.01	0.42	-2.5 – 2.2	*<0.001*
OC (ng/ml)	5.23	0.61	1.8 – 9.3	9.19	0.83	2.5 – 12.1	*<0.0001*
ALP (U/L)	209.5	6.1	178 – 255	319.5	13.3	263 – 383	*<0.0001*
Gal-Hyl (nmol/L)	61.18	1.2	53.4 - 70	110.29	1.4	103.1 –116.8	*<0.0001*
Calcium (mg/dl)	8.49	0.34	6.9 – 11.3	8.79	0.4	7.1 – 11.1	*>0.05*
Phosphorus (mg/dl)	3.45	0.19	2.2 – 4.9	3.11	0.09	2.8 – 3.7	*>0.05*
Dpd (nM/mM creat.)	6.27	0.17	5.5 – 8.4	11.5	0.6	8.1 – 13.5	*<0.0001*
Estradiol (pg/ml)	109.31	6	68 – 160	51.15	3.0	40 – 70	*<0.0001*

Non significant= P > 0.05; Significant = P < 0.05; Highly significant = P < 0.001; Very highly significant = P < 0.0001

Table 3. Comparison between osteopenic and osteoporotic groups regarding some investigated parameters

Sub-Group / Variable	Osteopenia (n= 12)			Osteoporosis (n= 10)			*p value*
	Mean	± SE	Range	Mean	± SE	Range	
Age	48.9	1.9	40 – 60	57.9	0.66	55 – 60	*<0.001*
Ap Spine BD %	82.9	0.96	77 – 88	75.8	1.2	72 – 86	*<0.001*
Ap Spine BD T score	-1.8	0.1	-2.3 - -1.5	-2.67	0.1	-3.2 - -2.5	*<0.0001*
Lt Femur BD %	94.9	2.6	73- 110	88.2	5.1	70 – 127	>0.05
Lt Femur T score	-0.29	0.22	-2.2 – 0.8	-1.01	0.42	-2.5 – 2.2	>0.05
OC (ng/ml)	9.28	0.59	6.4 – 13.1	9.19	0.83	2.5 – 12.1	>0.05
ALP (U/L)	275	5.8	257 – 331	319.5	13.3	263 – 383	*<0.05*
Gal-Hyl (nmol/L)	80.25	0.95	74.1– 86.8	110.29	1.4	103.1 –116.8	*<0.0001*
Calcium (mg/dl)	8.33	0.33	6.9 – 11.1	8.79	0.4	7.1 – 11.1	>0.05
Phosphorus (mg/dl)	3	0.81	2.3 – 3.5	3.11	0.09	2.8 – 3.7	>0.05
Dpd (nM/mM creat.)	7.9	0.19	7.1- 9.4	11.5	0.6	8.1 – 13.5	*<0.001*
Estradiol (pg/ml)	73.3	2.7	55 – 90	51.15	3.0	40 – 70	*<0.0001*

Non significant= P > 0.05; Significant = P < 0.05; Highly significant = P < 0.001; Very highly significant = P < 0.0001

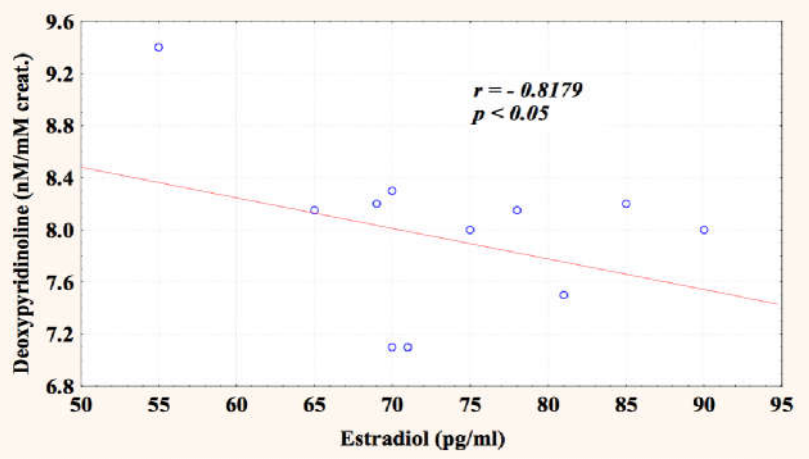

Fig. 3. Correlation between E2 and Dpd in osteoporotic patients

Moreover, urinary Dpd was obviously increased among the osteoporotic than the controls. No significant differences between the examined two groups were noticed in Ca and P levels. But for the E_2 value, a significant lower level was observed among the diseased group. Table (3) reveales a comparison between some laboratory data for the osteopenic and the osteoporotic groups. Obviously, a significant difference was observed as regard the age between both groups. While significant differences were observed as regards bone mineral density including Ap Spine BD % and Ap Spine BD T score. Both ALP and Gal-Hyl were significantly higher among the osteoporotic women when compared to the osteopenics. In addition, urinary Dpd was clearly increased among the osteoporotic subjects. Apparently, E_2 value showed a significant lower levels among the osteoporotic group. Figures (1, 2): illustrate the significant negative correlations between E_2 and OCin both osteopenic and osteoporotic groups respectively. Figure (3): shows the significant negative correlations between E_2 and urinary Dpd in osteoporotic patients.

DISCUSSION

Elevated bone turnover seems to be most pronounced in the early post menopause with a tendency to slow down with increasing age. This has been attributed not only to estrogen deficiency but also, to some extent to age-related increases of other hormones. The importance of estrogens in the metabolism of bone was reported by Peichl *et al*., (2004). Recent data suggested that estrogens play an important role not only suppressing bone resorption, but also as physiological regulators of osteoblastic activity (Johansson *et al.,* 2004). Yilmaz *et al*., (Bouxsein *et al.,* 1998) verified that very low serum E_2 level in post-menopausal women has been associated predominantly with enhanced bone resorption and to a lesser degree bone formation as well as more rapid bone loss. Sypniewska and Chodakowska-Akolinska (Black *et al.,* 1992) reported that bone markers might be used to predict osteoporotic fractures in postmenopausal women and to monitor anti-resorptive therapy. They concluded that measurement of serum biochemical estimates of bone turnover might be of clinical value to identify groups at higher risk of osteoporosis. Biochemical markers of bone turnover allow clinicians to evaluate the risk of bone loss and provide insight into response to therapy (Siris *et al.,* 2001).

The results of the present study revealed significant higher levels of OC in both osteopenic [(mean ± SE): (9.28 ± 0.59 ng/ml)] and osteoporotic groups (9.19 ± 0.83 ng/ml) compared to the controls (5.23 ± 0.61 ng/ml), but no significant difference was detected between the osteopenic and the osteoporotic groups. These results agreed with that of Lee *et al.,* (Blake, 2001) who noticed that the serum OC level was 10% higher in postmenopausal osteoporosis. This study showed the presence of significant negative correlation between OC and BMD in osteoporotic women (OC and AP%: r = -0.9091 at p< 0.001andOC and left femur % r = -0.9613 at p< 0.0001).Thus, this came in accordance with that obtained by Yasumura *et al*., (Kannus *et al.,* 2006) which might indicate that higher rates of bone turnover could be associated with more rapid bone loss in osteoporotic women. Minosola (Leslie *et al.,* 2009) found no correlation between BMD and OC. This could be explained on the fact that their patients were younger than ours. The foregoing results of this study presented a significant increase of serum ALP in both the osteopenic (275 ± 5.8 U/L) and osteoporotic (319.5 ± 13.3 U/L) comparing them with normal women (209.5 ± 6.1 U/L), also a significant difference ($p<0.05$) was detected on comparing the diseased groups together. These results could be elucidated with those found by Dominguez *et al*., (Eastell *et al.,* 1989). The increased levels of the two indices of bone turnover, ALP and OC suggested that the mean bone turnover was higher in osteoporotic women (Jaglal *et al.,* 2005). This finding could explain the significant positive correlation between OC and ALP in the osteoporotic group ($r = 0.6937$ at $p< 0.05$). On the other hand, some investigators (Nayak *et al.,* 2006) found no correlation between OC and ALP among osteoporotic patients. The possible explanation was that ALP is a rough index of osteoblastic activity and non-osseous tissues might be considered as a serum pool of this enzyme. Serum Gal-Hyl could be considered as a most sensitive biochemical marker of bone resorption, our results showed significant increases in both osteopenic (80.25 ± 0.95 nmol/L) and osteoporotic (110.29 ± 1.4 nmol/L) patients versus the control group (61.18 ± 1.2 nmol/L). Also, a high significant increase was detected in the osteoporotic patients compared to the osteopenics ($p<0.0001$).

In addition, a significant positive correlation between OC and Gal-Hyl was found in the osteoporotic women. *Abdul Wahed et al*., (Cummings *et al.,* 1995), measured Gal-Hyl in the serum and urine by HPLC and they concluded that the concentration of Gal-Hyl in both serum and urine discriminated between pre-menopausal women, pubertal girls and patients with untreated Paget's disease. Increased levels of Gal-Hyl reflects the high bone turnover (Davis *et al.,* 1994). As regards urinary Dpd, the present study demonstrated that the mean urinary Dpd level was significantly increased in osteopenic (7.93 ± 0.19 nM/mM Creat.) and osteoporotic women (11.5 ± 0.6 mM/mM Creat.) against the controls (6.27 ± 0.17 nM/mM Creat.). Again, a high significant difference was obtained when comparing the osteopenic against the osteoporotic groups ($p< 0.0001$). Our data revealed a significant positive correlation between OC and Dpd ($r = 0.8817$ at $p< 0.001$). Yilmaz *et al.,* (Black *et al.,* 1992) clarified that Dpd was significantly high in osteoporosis and its concentration increased with the severity of the disease. Lee *et al.,* (Kanis *et al.,* 2006) reported that bone resorption markers are more efficient than bone formation markers.

They added that urinary Dpd level was 50% higher in postmenopausal osteoporosis than in premenopausal women. Regarding Ca and P levels, the results of the current study revealed no significant difference in both Ca and P levels in cases of osteopenia (8.33 ± 0.33 mg/dl for Ca and 3 ± 0.8 mg/dl for P) and osteoporosis (8.79 ± 0.4 mg/dl for Ca and 3.11 ± 0.09 mg/dl for P) when compared to the controls (8.49 ± 0.34 mg/dl for Ca and 3.45 ± 0.19 mg/dl for P). This came in accordance to the result of Yilmaz *et al.,* (Black *et al.,* 1992) who reported that the serum Ca and P did not show any significant difference between normal and osteoporotic women. Moreover, the current data revealed a significant negative correlation between OC level and serum Ca in osteoporotic women ($r = - 0.9048$ at $p< 0.001$). These findings pointed out that the accelerated skeletal turnover rate, as shown by the high OC level was associated with a net loss of bone (Melton *et al.,* 1993). A point of interest in this study is the significant decrease in E_2 level in osteopenic (73.3 ± 2.7 pg/ml) and osteoporotic women (51.15 ± 3 pg/ml) when compared to the controls (109.31 ± 6 pg/ml). Estradiol level was significantly inversely correlated with OC in both

osteopenic (r= -0.9036 at p< 0.0001) and osteoporotic women (r= - 0.7508at p< 0.01). Also, a significant negative correlation was detected between urinary Dpd and E_2 (r= - 0.8179 at p< 0.05) in the osteoporotic group. As well, a non-significant negative correlation was identified between Gal-Hyl and E_2 in the same group. All of these results could be explained on the fact that E_2 plays an important role not only suppressing bone resorption but also, as physiological regulators of osteoblastic activity (Eastell*et al.,* 1989).

So, a very low serum E_2 level in postmenopausal women has been associated predominantly with enhanced bone resorption, measured by biochemical markers (Gal-Hyl) and to a lesser extent bone formation (Kanis and Johnell, 2005) as well as rapid bone loss (Cummings *et al.,* 1995). It could be concluded that the decreasing level of E_2 in early and post-menopausal women is the main cause of osteoporosis. The measurement of serum and urinary estimates mainly serum Gal-Hyl may be of clinical value to identify groups at higher risk of osteoporosis and to predict bone loss.

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