

**Study Some Physiological Parameters and Association  
*GSTM1* , *GSTT1* Genes in Patients Osteoporosis in AL-  
Diwanya City.**

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**Abstract:**

Several studies refer to higher levels of oxidative stress are associated with osteoporosis .The glutathione S- transferase genes have a vital role in the protection of cells from oxidative stress.

The purpose of this study to know the frequency of *GSTM1* and *GSTT1* genes in Osteoporosis patients and asset the relationship between the genes and some physiological parameters in osteoporosis patients. Genotype (*GSTM1* and *GSTT1*) genes were determined by polymerase chain reaction (PCR).

The mean Calcium and GSH level were significantly lower ( $P<0.05$ ) in osteoporosis patients compared with the control group.

Show the results significantly higher ( $P<0.05$ ) levels ALP, Phosphorus and MDA in osteoporosis patients compared with the control group. While don't find significantly higher ( $P>0.05$ ) GST in osteoporosis patients compared with the control group.

The statistical analysis showed that the deletion in the *GSTM1* (-) null genotype was increased (3.4) times in osteoporosis patients compared with control. While, the deletion in *GSTT1* (-) null genotype was increased (1.5) times in osteoporosis patients. Also the results found that significant increased in the deletion in *GSTM1* (-) and *GSTT1* (-) (2.9) times compared with the control group.

**Introduction**

Bone metabolism is a dynamic and continuous process to maintain a balance between the resorption of old and injured bone initiated by osteoclasts and the formation of new bone under the contral of osteoblasts (1).Through childhood and early adulthood, formation exceed resorption so that bone density increase and then plateaus until the age of 30 to 40 years. After that, resorption exceeds formation and bone density decrease through the rest of life, Which in turn lead to osteoporosis (2). Osteoporosis is a bone disease characterized by low bone mineral density (BMD) with high risk of fractures . It occurs when there is an imbalance between bone resorption and bone formation during the bone remodeling process.

Free radicals play an important role in bone remodeling by promoting differentiation and bone resorptive activity of osteoclasts. However, high levels of free radicals may lead to oxidative stress and need to be converted to less reactive forms by antioxidant enzymes (3).

All the various risk factors of Osteoporosis listed a bove were linked to oxidative stress. Risk factors of osteoporosis such as smoking (4), hypertension (5) and diabetes

mellitus (6) were all associated with oxidative stress. Other negative effects in the oxidative stress model included deterioration of the structural, static and dynamic parameters of bone histomorphometry(7 , 8) impairment of bone mineralization (9) and decreased bone calcium content (10).

Free radicals , when they one out of balance with antioxidant capacity, have been suggested to be involved in the pathogenesis of osteoporosis by modulating the differentiation and survival of osteoblasts (11) and by stimulating bone resorption (12), inflammation, resulting from reactive oxygen species (ROS), has also been recently connected to osteoporosis (13).

The glutathione S- transferase (GSTs) represents an important super family of phase II xenobiotic metabolizing enzymes that catalyze the conjugation of reduced glutathione and those potentially hazardous reactive electrophilic species to detoxify them and facilitate their excretion (14, 15), highly expressed in liver and also in bone cells (16). They are involved in protection against chemical toxins, many xenobiotic agents and the products of endogenous lipid peroxidation and inactivating organic hydroperoxides (17, 18, 19). They have many functions as disease modifying enzymes when oxidative stress is a contribution (19). The GSTs polymorphisms are also associated with the organism phase II detoxification capacity and can help in identifying subgroups that are susceptible to many age- related disease , such as some types of cancer (20).

Human GSTs are divided into cytosolic, microsomal and mitochondrial families (21). Because of the polymorphism of gene deletion, our study is focused on only two genes, *GSTM1* and *GSTT1* form two classes of the cytosolic family GST Mu (*GSTM1* ; Chromosome 1p13.3) and GST Theta (*GSTT1*; Chromosomes 22p11.2), respectively. These polymorphisms depend on the gene deletion, finally resulting in a lack of protein , this leading to functional deficiency in the enzyme activity of circulating red cells (22, 23).

Two GSTs *GSTM1* and *GSTT1* play an important role in cellular protection by conjugation several environmental carcinogens, endogenous compounds such as peroxidized lipids and inactive products formed as secondary metabolites during oxidative stress, with endogenous ligands to facilitate their excretion (17, 18).

## **Materials and methods**

### **collection of samples:**

This study involved (30) Osteoporosis patients (11 male, 19 female) from the Department of Fraction and Bone in AL- Qadisiya hospital / Iraq. The mean age of the patients was (40-70 year). The control group was composed of (30) health volunteers (14 male, 16 female ) with a mean age of ( 39 -70 year ). Controls had no medical problems.

The blood sample was collected from the Osteoporosis patients directly from arteriovenous fistula. The blood sample was divided into two tubes (with and without anticoagulant- EDTA )

Blood was collected by EDTA, then stored at -20 C° till used for DNA extraction.

### **Isolation of genomic DNA:**

Genomic DNA was isolated by DNA extraction kit (Genedia DNA Mini Kit , UKAS) and stored directly at 4 C till used.

### **Polymerase Chain Reaction (PCR):**

The *GSTM1* and *GSTT1* genotype were analyzed by multiplex PCR according to the protocol of (24). Genomic DNA was amplified by using six sets of primers table (1) .

**Table 1:Oligonucleotide primer sequences used for PCR**

Primers		Primer Sequences	Length	MT	AT
<i>GSTM1</i>	* F	5'-GAA CTC CCT GAA AAG CTA AAG	22	64 °	59 ° C
	*R	C-3 5'-GTT GGG CTC AAA TAT ACG GTC	22	C 64 °	59 ° C C
<i>GSTT1</i>	F	5'-TTC CTT ACT GGT CCT CAC ATC	22	64 °	59 ° C
	R	TC-3 5'-TCA CCG GAT CAT GGC CAG CA-3	22	C 64 °	59 ° C C
<i>Albumin</i>	F	5'-GCC CTC TGC TAA CAA GTC CTA	22	64 °	59 ° C
	R	C-3 5'-GCC CTA AAA AGA AAA TCG CCA	22	C 64 °	59 ° C C

**amplification of *GSTM1*, *GSTT1* genes**

Albumin was used as a control. MT : Melting temperature AT: Annealing Temperature

\* F : Forward

\* R : Reverse

**The reaction mix and PCR condition are given in table ( 2, 3).**

**Table 2: The reaction mix (25µl) for *GSTM1* and *GSTT1* genes**

Chemicals	Volume
Master Mix	12.5 µl
Primer Forward	1 µl
Primer Reverse	1 µl
DNA	5 µl
D.W.	5.5 µl
Total	25 µl

**Table 3:PCR conditions for *GSTM1* and *GSTT1* genes**

NO. OF STEPS	STEPS	TEMPERATURE	TIME	NO. OF CYCLE
1	Denaturation 1	95 ° C	3 min	1 Cycle
2	Denaturation 2	95 ° C	1 min	30 Cycles
3	Annealing	58 ° C	1 min	
4	Extension 1	72 ° C	1 min	
5	Final Extension	72 ° C	5 min	1 Cycle

The PCR product was than subjected to electrophoresis on a 2% agarose gel. The presence of bands of 480 and 215 bps was indicative of the *GSTT1* and *GSTM1* genotypes; whereas the absence indicated the null genotypes for that gene. *Albumin* indicated by a 350 bp product was used as an internal control.

Blood were collected by non- EDTA tubes was centrifuged at 2000 rpm for 20 minutes, The clear supernatants serum were frozen till the time of hormones and

biochemical estimations including the levels of Calcium, Phosphorus , ALP, these were measured using an automatic analyzer (Reflotron ) (Germany). Plasma GSH and MDA levels were determined respectively by the method of (25), assay the activity of serum GST by according to the method (26).

### **Statistical Analysis :**

The data were analyzed by using students T- test taking ( $P < 0.05$ ) as the lowest limit of significant of difference .

$X^2$  tests were used to examine differences of allele and genotype frequencies between patients and controls. Fisher's exact test was used. ORS and The 95 % CI were calculated and ( $P < 0.05$ ) considered signification (27).

### **Result**

The mean age of Osteoporosis patients was ( $59.91 \pm 2.25$ ) years than ( $48.25 \pm 4.16$ ) years of control, There was significant difference ( $P < 0.05$ ) in age of control and patients. The mean duration of osteoporosis patients was ( $8.73 \pm 0.13$ ) month. Table (4).

Show gender in osteoporosis patients male (%36.66) and female (%63.33) comparative control (%46.66) male and (%53.33) female.

The results in table (4) show that there is significantly increased ( $P < 0.05$ ) in serum ALP enzyme patients Osteoporosis ( $75.11 \pm 2.04$ ) compared to those of healthy subjects ( $64.19 \pm 3.10$ )

The statistical analysis of data shows in the table (4) show the there was a significantly decrease ( $P < 0.05$ ) in serum calcium level in patients ( $1.81 \pm 0.82$ ) comparative the control ( $2.41 \pm 0.51$ ), While the show was significantly increased ( $P < 0.05$ ) in serum Phosphorus of Osteoporosis patients ( $4.15 \pm 0.51$ ) comparative level Phosphorus of control ( $1.36 \pm 0.42$ ) .

Table (4) Clearly shows that plasma GSH was a significant decrease ( $P < 0.05$ ) in Osteoporosis patients ( $0.97 \pm 0.02$ ) when compared to control ( $1.75 \pm 0.82$ ). While the data presented in table (4) show a significant increased ( $P < 0.05$ ) in serum MDA of Osteoporosis patients ( $8.12 \pm 3.86$ ) than control health ( $3.21 \pm 0.10$ ).

The result of serum GST enzyme in osteoporosis and control are shown in table (4) that there is no significant ( $P > 0.05$ ) difference level GST in patients was ( $3.05 \pm 1.59$ ) than in control was ( $2.93 \pm 0.31$ ).

**Table 4: Distribution of Demographic variables of the osteoporosis patients and controls.**

Parameters	Control (n = 30) ( Mean ±SD)	Patients (n = 30) ( Mean ±SD)
Age (Years)	48.25 ± 4.16	59.91 ± 2.25
Gender (Male)	14 (% 46.66)	11 (%36.66)
(Female)	16 (% 53.33)	19 (%63.33)
Duration of Osteoporosis (Month)	-----	8.73 ± 0.13
ALP (U/I)	64.19±3.10	* 11.75±2.04
Calcium (mmol/L)	2.41±0.51	* 1.81±0.82
Phosphorus (mmol/L)	1.36±0.42	* 4.15±0.51
GSH (µmol /dl)	1.75 ± 0.82	*0.97 ± 0.02
MDA (nmol /ml)	3.21 ± 0.10	* 8.12 ± 3.86
<b>GST (nmol /min/mg)</b>	<b>2.93 ±0.31</b>	<b>3.05 ± 1.59</b>

\* Significant difference between osteoporosis and control (P< 0.05).

The frequencies of *GSTM1*- null genotypes were (43.3%) in the Osteoporosis patients and (16.6%) in the control, the difference being statistically significant (P= 0.031, OR= 3.8, 95% CI=15.0-17.14).The frequency of the *GSTT1*- null genotype was (40%) in the Osteoporosis patients and (30%) in control. The difference was significant(P= 0.015, OR= 1.5, 95% CI=24.8-36.8)

The combinations of *GSTM1*- and *GSTT1*- null genotype was (40%) in the Osteoporosis patients and (16.6%) in the control, the difference was significant (P=0.038, OR=2.9, 95% CI= 22.1-31.3). Shown table (5).

**Table 5: Distribution of polymorphism of *GSTM1* and *GSTT1* gene among osteoporosis patients and controls.**

Genotypes	Control (n= 30)	Patients (n= 30)	OR	P-CI95%	P-Value
<i>GSTM1</i> (+)	25 (% 83.3)	17(% 56.6)	1.0		
<i>GSTM1</i> (-)	5 (% 16.6)	13 (% 43.3)	3.8	15.0-17.14	0.031
<i>GSTT1</i> (+)	21 (% 70)	18(% 60)	1.0		
<i>GSTT1</i> (-)	9 (% 30)	12(% 40)	1.5	24.8-36.8	0.015
<i>GSTM1,GSTT1</i> (+)	21 (% 70)	17(% 56.6)	1.0		
<i>GSTM1,GSTT1</i> (-)	5 (% 16.6)	12(% 40)	2.9	22.1-31.3	0.038

*GSTM1* (+) , *GSTT1* (+) Present      *GSTM1* (-) , *GSTT1* (-) Null

OR: Odds ratio

CI : Confidence interval from binary logistic regression.

Show the results in a table (6) levels of ALP and Phosphorus were significant increase ( $P < 0.05$ ) with the genotypes of *GSTM1*- and *GSTT1*- than those with the genotypes of *GSTM1*+ and *GSTT1*+ , but the levels Calcium shows significant decrease ( $P < 0.05$ ) with the genotypes of *GSTM1*- and *GSTT1*- than those with the genotypes of *GSTM1*+ and *GSTT1*+.

The Levels of GSH were significantly lower in those with the genotypes of *GSTM1*- and *GSTT1*- than those with the genotypes of *GSTM1*+ and *GSTT1*+ , While levels of The Levels of MDA were significantly higher in subjects with the genotypes of *GSTM1*- and *GSTT1*- than those with the genotype of *GSTM1*+ and *GSTT1*+ . That shown in table (6).

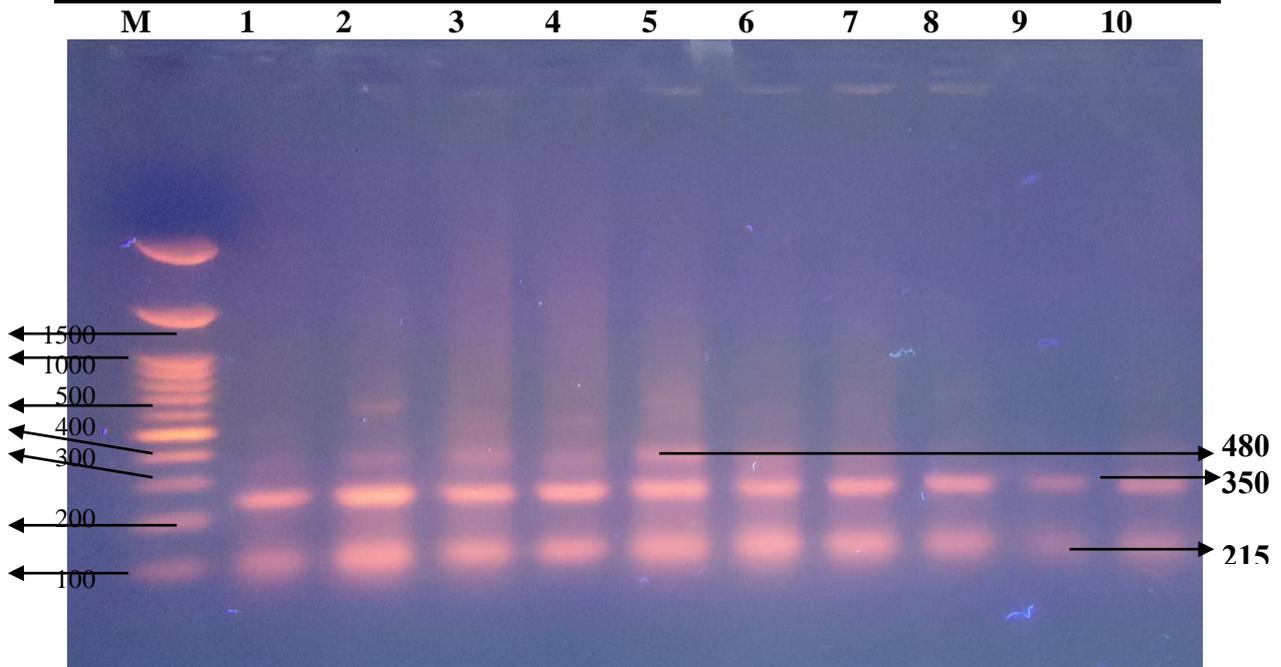
The statistical analysis of data shows in the table (6) there was no significant increase ( $P > 0.05$ ) level of GST with the genotypes of *GSTM1*- and *GSTT1*- than those with the genotypes of *GSTM1*+ and *GSTT1*+.

**Table 6: Association between combination of *GSTM1* and *GSTT1* genotype among and biochemical parameters in osteoporosis patients.**

Genotypes Parameters	<i>GSTM1</i> (+) (n = 17)	<i>GSTM1</i> (-) (n = 13)	P- Value	<i>GSTT1</i> (+) (n = 18)	<i>GSTT1</i> (-) (n = 12)	P- Value
ALP (U/I)	63.11±2.10	75.09±1.06	0.374	60.19±2.10	41.65±1.04	0.374
Calcium (mmol/L)	2.11±0.61	1.82±0.32	0.514	2.01±0.51	1.11±0.22	0.870
Phosphorus (mmol/L)	1.38±0.42	3.15±0.51	0.291	1.66±0.42	3.85±0.61	0.674
MDA (nmol /ml)	3.12 ± 1.57	8.43 ± 3.49	0.674	3.62 ± 1.33	7.45 ± 1.54	0.311
GSH (µmol /dl)	1.53 0.13	0.64 ± 0.22	0.061	± 0.759 1.44	± 0.173 0.318	0.073
GST (nmol /min/mg)	2.03 ±0.36	2.48 ± 0.21	0.072	± 1.23 2.84	± 1.60 3.10	0.080

*GSTM1* (+) , *GSTT1* (+) Present      *GSTM1* (-) , *GSTT1* (-) Null

Genotypic analysis of the samples using a multiplex PCR to analyze simultaneously the presence or absence of *GSTM1* and *GSTT1*. The *Albumin* gene was used as an internal control. The internal control amplified *Albumin* fragment was 350bp in length, whereas the presence of the *GSTM1* and *GSTT1* genes were identified by 215 and 480 bp fragments, respectively.

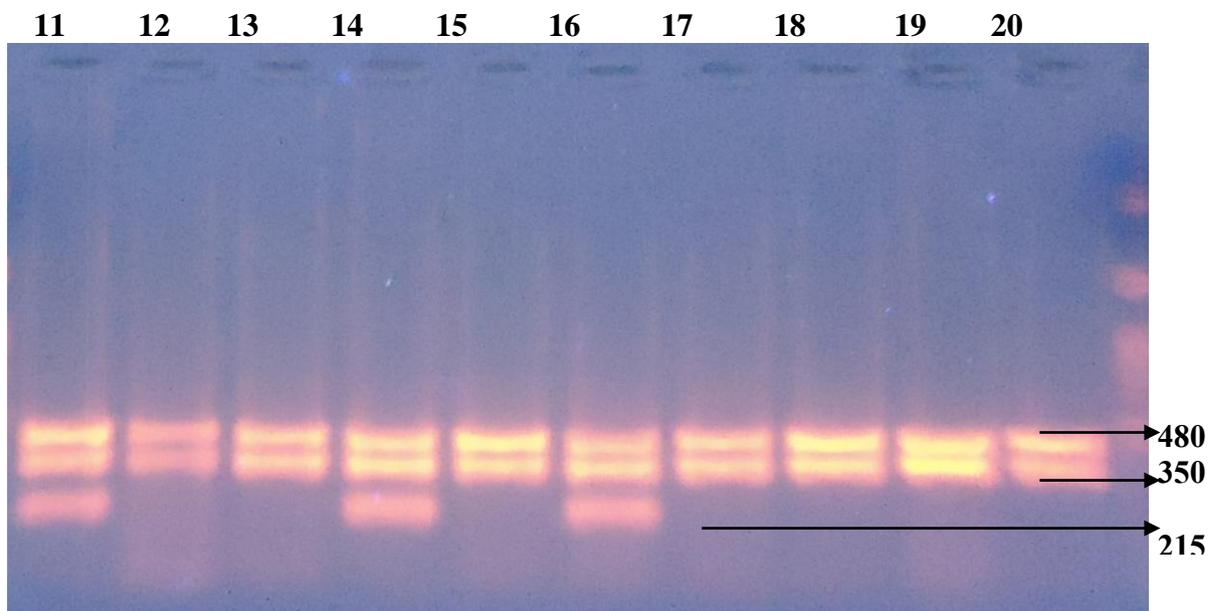


**Fig 1: Electrophoresis of PCR products on agarose gel 2% .**

Line M= DNA marker.

Line 2,3,4,5 Normal genotype (GSTM1 + and GSTT1 + )

Line 1, 6, 7, 8, 9, 10 *GSTT1* - null genotype.



**Fig 2: Electrophoresis of PCR products on agarose gel 2% .**

Line 11, 14, 16 Normal genotype ( GSTM1 + and GSTT1 +)

Line 12, 13, 15, 17, 18, 19, 20 *GSTM1* - null genotype.

## **Discussion**

Osteoporosis is disease diagnosis old than the age of 40 years, The maximum number of patients in this study were in the age group of ( 48-61) years, and the mean age was  $(59.91 \pm 2.25)$ . Osteoporosis and age related bone loss is associated with changes in bone remodeling characterized by decreased bone formation relative to bone resorption, resulting in bone fragility and increased risk of fractures(30).Through childhood and early adulthood formation exceeds resorption so that bone density increases and then plateaus until the age of (30 – 40) years. After that, resorption exceeds formation and bone density decrease through the rest of life, which in turn may lead to osteoporosis (2).

The gender percent of were diagnosed with osteoporosis in male (%36.66) and in female (%63.33). That's because the osseous mass of men is greater than women, osteoporosis occur in men 10 years later than in women (31).

The results show Osteoporosis is a metabolic bone disease affecting both men and women especially postmenopausal (32). Bone loss begins in mid life in both women and men , but women undergo a phase of rapid bone loss in the decade the menopause

Biochemical markers of bone turnover have been shown to provide valuable information for the diagnosis and monitoring of metabolic bone disease (33). They reflect the whole body rates of bone resorption.

Our results of this study show that there is increased level ALP enzyme in osteoporosis patients, these results seem to agree with those of other investigations (34, 35). This enzymes play an important role in bone formation, the ALP activity is greater in increased in any disease characterized by an increased bone formation (36). Bone specific ALP is increasing in metabolic bone disorders like osteoporosis osteomalacia and rickets (37).

On the other hand, the results in osteoporosis patients show decreased level in serum Calcium, While the increase level Phosphorus. Calcium is a very important nutrient (38) and inadequate calcium intake (deficiency of calcium) or poor intestinal absorption gives rise to PTH mediated increased in bone resorption, and it is our of several important causes of reduced bone mass and osteoporosis (39). In chronic hypocalcaemia that results in a failure of the bone to mineralise normally, rickets in growing children (40).

Through a complex physiological system comprising the interaction of the calcitropic hormones, such as parathyroid hormones which increases the plasma calcium, calcitonin which decreases the plasma calcium and 1,25 (OH) 2 D3 which promotes the absorption of calcium by the intestine and its movement into and out of bone (41).

In osteoporosis an important role has sufficient intake of calcium as well as its optimal utilization by the organism. A number of factors influence the absorption of calcium as well as its excretion in urine (42). Absorption depends on the chemical form of calcium, the presence of another substance in food, physiological factors, the amount of calcium in the diet and timing of its ingestion during the day (43). One of substances which influence absorption of calcium most significantly its vitamin D, which increases its intestinal absorption and thus can within limits, compensate for its lesser intake (43).

Diet low in protein is a risk factor for osteoporosis it reduces calcium absorption and accelerates demineralization of bone in older age, particularly of vertebrae and the neck of femur. Amino acids with SH- group which come predominantly from animal protein, reduce urinary pH, thus reducing reabsorption of calcium in renal tubules,

This negative effect has however no significant bearing on the risk of osteoporosis (42).

Both calcium and phosphorus are required for the appropriate mineralization of the skeleton and a depletion of serum phosphate leads to impaired bone mineralization and compromised osteoblast function. Deformation of bone is repaired by osteoblast with the release of the enzyme ALP which helps in mineralization of bone by deposition of calcium phosphate (44).

The statistical analysis of data shows that there is a decrease in the antioxidant status level in serum GSH with no significance in level GST, While the increase level in serum MDA in osteoporosis patients.

osteoporosis subjects were found to have low antioxidants (45) and high levels of reactive oxygen radical (ROS) (46), meaning that they were under increased oxidative stress. However, high levels of free radicals may lead to oxidative stress and need to be converted to less reactive forms by antioxidant enzymes (3). GSH is an antioxidant that protects cells from toxins such as free radicals. This result agrees with results (47) that show decrease in level GSH in osteoporosis patients. Some investigations have indicated that osteoporosis are associated with biochemical markers of oxidative stress such as urinary excretion of isoprostanes and reduction of plasma antioxidants (48). The ROS are neutralized by the antioxidant system in the body, osteoblasts produce antioxidants such as GSH to protect against ROS (49).

Osteoporosis due to oxidative stress results in excessive free radical formation indicated by the increased MDA level (50). Level MDA is increase in patient suffering from osteoporosis, it is in accordance with previous findings that in osteoporosis decreased in osteoblastic activity leads to increase in osteoclastic activity (cells responsible for bone resorption) resulting in excessive free radical formation and these reactive oxygen species degrade polyunsaturated lipids, forming malondialdehyde. This compound is a reactive aldehyde, production of MDA is used as a biomarker to measure the activity of osteoclast in osteoporosis patients (50). Low levels of enzymatic antioxidants are also frequently implicated in the pathology of osteoporosis (45). This suggests that variation in host effectiveness in detoxifying ROS activity products could be important. The widely expressed glutathione S- Transferase (GST) super gene family appear to be critical in the protection of cells against (51). GST protein catalyzed the conjugation of electrophiles to GSH, charges in protein level due to variation at the genetic level many modulate the oxidative damage or inflammation caused by environmental exposure. Therefore, individual susceptibility to toxic agents may vary with GST polymorphisms (52, 53).

GST substrates also include ROS products of endogenous lipid peroxidation and inactivating organic hydroperoxides including thymine hydroperoxide and arachidonic acid hydroperoxide, which may arise from inflammation. Oxidative stress causes alterations redox balance by affecting changes in the GSH: GSSG ratio which in turn influences expression of immunological and bon relevant transcription factors (54, 55).

The GST family of genes is critical in the protection of cells from ROS because they utilize as substrate a wide variety of product of oxidative stress. *GSTM1* and *GSTT1* demonstrate activity towards phospholipids hydroperoxide (56). Furthermore, the ROS derived products are essential in the mobilization of arachidonic acid, with subsequent production of pro-inflammatory eicosanoids (56).

There are few studies on the relation between GST genotypes and osteoporosis . There are data on their potentiation of risk of cytogenetic damage in many diseases, only osteoporosis association study on deletion polymorphism of *GSTM1* and *GSTT1*

genes has been reported; it showed no significant association with bone density values in an elderly Japanese population (57).

In our study the significant association of *GSTT1* gene deletion variant, alone or in combination with *GSTM1* gene deletion variant, add to the list of antioxidative enzyme gene polymorphisms associated with BMD value. *GSTM1* and *GSTT1* have the null genotype that is characteristic of no enzyme activity, reduced inactivation of oxidative stress was observed in people with *GSTM1* null genotype (58). Epidemiological studies proposed that *GSTM1* and *GSTT1* null genotype were correlated with an increased susceptibility to disease associated with oxidative stress (59, 60).

Alteration of *GSTM1* result in a failure to express the *GSTM1* gene in 50% - 60% of individuals (61). Although *GSTM1* expression is concentrated in the liver, it is involved in the conjugation (and thus transport and excretion) of a broad range of antibiotics and xenobiotics from different tissues (62) possibly also influence gene expression in different tissues.

The GST family is involved in the conjugation and thus excretion of toxic oxidant production, which are increased in osteoporotic people (17, 18) polymorphisms or deletion with these genes alter the catalytic activity of the GSTs to varying extents. The study by (63) showed that, under oxidative stress induced by H<sub>2</sub>O<sub>2</sub>, the highest and lowest extents of DNA damage depended on the interaction between *GSTM1* and *GSTT1* polymorphisms.

Contrary to the results presented here, it has been reported that *GSTM1* and *GSTT1* gene deletion genotype do not significantly affect susceptibility to osteoporosis in Japanese osteoporotic patients assessed using ultrasound bone tissue measurement (57).

In human osteoblasts, *GSTM1* is 2- times more expressed than a *GSTT1* gene, consequently resulting in 2- times more, effective detoxification in bone cells (64).

Both genes have been considered to encode carcinogen metabolizing enzymes implying they mediate the detoxification of potential mutagens such as polycyclic aromatic hydrocarbons, which are found in many common exposures such as cigarette smoke, diesel fume and grilled meats (65).

ROS are known to be produced by osteoclast bone cells during the bone remodeling process, causing oxidation of DNA and lipids and giving rise to a variety of cytotoxic products, including lipid and DNA hydroperoxides and alkenals. ROS also mediate the bone remodeling process that result in osteoporosis (12).

*GSTM1* and *GSTT1* genes are polymorphic in human and the null genotypes lead to the absence of enzyme function, contributing to inter individual differences in response to xenobiotics (66). A deletion polymorphism resulting in a total lack of enzyme activity in the homozygous null genotype, affects both *GSTM1* and *GSTT1*.

In this study, we found that, among osteoporosis patients, The *GSTM1* and *GSTT1* genotype were associated with a significantly lower antioxidant capacity in terms of blood GSH, GST compared with *GSTM1*+, *GSTT1*+ genotype. Glutathione is a major intracellular antioxidant, and a decrease in the whole blood level provides insight into a defective cellular redox state. We found that the lack of GST enzymes activity contributes to the augmented oxidative stress and that this subsequently leads to a further decrease in the levels of GSH among in osteoporosis patients.

The present study was conducted to look into the relationship between the genetic polymorphism of glutathione S- transferase *GSTM1*, *GSTT1* gene and osteoporosis which is involved in the metabolism of ROS and detoxifying xenobiotics. The contribution of the GST super gene family to oxidative stress resistance is well established (67) and therefore the absence of one or more of GST enzymes would

result in increased ROS. *GSTM1* and *GSTT1* demonstrate activity towards phospholipids hydroperoxide (56). It has been reported that an individual difference in metabolic activation and detoxification xenobiotics partly depends on the genetic polymorphisms associated with *GSTT1*, *GSTM1* enzymes.

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## دراسة بعض المعايير الفسيولوجية ومرافقة الجينيين *GSTT1* و *GSTM1* في مرضى هشاشة العظام في مدينة الديوانية

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### الخلاصة:

أشارت العديد من الدراسات المستويات العالية من الإجهاد التأكسدي تترافق مع مرض هشاشة العظام . جينات glutathione S- transferase genes (GST) تمتلك دور حيوي في حماية الخلايا من الإجهاد التأكسدي. الغرض من هذه الدراسة لمعرفة تردد الجينيين *GSTT1* و *GSTM1* في مرضى هشاشة العظام وتقييم العلاقة بين جينيين وبعض المعايير الفسلجية لمرضى هشاشة العظام. حددت الطراز الوراثي لجينيين *GSTM1* و *GSTT1* بتقنية تفاعل البلمرة التسلسلي المتعدد PCR. وجد انخفاض معنوي ( $P<0.05$ ) لمعدل مستوى الكالسيوم و الكلوتاتايون GSH في مرضى هشاشة العظام مقارنة مع مجموعة السيطرة. بينت النتائج ارتفاع معنوي ( $P<0.05$ ) في مستوى أنزيم الفوسفاتيز ALP والفسفور و المالنوالديهايد MDA في مرض هشاشة العظام مقارنة مع السيطرة. بينما وجد ارتفاع لم يصل إلى مستوى المعنوية ( $P>0.05$ ) في مستوى أنزيم الكلوتاتايون ترانسفيريز GST لمرضى هشاشة العظام مقارنة مع مجموعة السيطرة. أظهرت نتائج التحليل الإحصائي إن فقدان الجين *GSTM1* ازداد بمقدار (3.8) لدى مرضى هشاشة العظام مقارنة بمجموعة السيطرة. بينما ازداد فقدان الجين *GSTT1* بمقدار (1.5) مرة في المرضى. كذلك أظهرت النتائج زيادة معنوية ( $P<0.05$ ) في فقدان الجينيين *GSTT1* و *GSTM1* عند مرضى هشاشة العظام بمقدار (2.9) مقارنة بمجموعة السيطرة .