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The Potential Regulatory Role of miRNA 185-5p in the Expression of Glutathione Peroxidase 1 *GPX1* in Men with Primary Osteoporosis

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Abstract: Primary osteoporosis in men remains a poorly understood but clinically important condition, marked by weakened bones and a higher likelihood of fractures. This study included 60 blood samples collected from 30 Iraqi men diagnosed with primary osteoporosis and 30 healthy control men. To assess the concentration of biological parameters (Alkaline Phosphatase (ALP), calcium, and vitamin D), and investigate the molecular changes involving the *GPX1* gene expression and miR-185-5p in men with osteoporosis and healthy control. Serum ALP, calcium, and vitamin D levels were measured, while qPCR analyzed *GPX1* and miR-185-5p expression. The findings showed that men with osteoporosis had significantly lower BMD, T-scores, reduced calcium and vitamin D deficiency and higher ALP compared to healthy controls. The cycle threshold (Ct value) of *GPX1* in the patients (mean ±SD 22.36± 0.26) compared with the Ct value of healthy control group (mean ± SD 22.2±0.45). Meanwhile, the Ct value of miRNA185-5p in primary osteoporosis patients was elevated (mean ± SD 29.36±0.65) while the control group (mean ± SD 29.00±0.7). These results suggest that oxidative stress and dysregulated miR-185-5p may play key roles in bone loss in men, beyond other risk factors. The study identifies potential new biomarkers and therapeutic targets, shedding light on the complex genetic, and biochemical interactions in male osteoporosis.

Keywords: BMD, bone biomarkers, *GPX1*, miR-185-5p, osteoporosis in men, oxidative stress.

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Introduction

Osteoporosis is a disease that effect all populations' regardless ethics, gender and age various studies clarified the percentage occurrence of osteoporosis in many countries and continents (1). The majority of Osteoporosis patients are postmenopausal women, however also affects men about (12% globally) compared to women (25–30%) but still studies concerning men are limited (2). Bone Mineral Density (BMD) measures calcium and mineral levels in bones, with lower BMD levels the risk of fractures increase. Osteoporosis, defined by WHO, when BMD is equal 2.5 or more below the mean peak for healthy individuals (1). Nevertheless, bone health is influenced by a variety of additional factors, underscoring the multifaceted nature of maintaining healthy bone mineral density (BMD). Calcium serving as a fundamental building block of bone and vitamin D playing a critical role in calcium absorption and regulation. Also vitamin D, helps maintain optimal blood calcium levels. Furthermore; alkaline

phosphatase (ALP), an enzyme involved in bone metabolism, acts as a key indicator of bone formation and turnover. Elevated ALP levels often reflect on the bone remodeling activity that observed in osteoporosis. Together, these parameters used as indicators for bone growth, remodeling, and the dynamic balance between osteoblasts and osteoclasts, thereby help in diagnosing osteoporosis (3-4). Glutathione peroxidase enzyme is an antioxidant enzyme presents in all cells, the mitochondria and cytosol ubiquitously, and plentiful expressed in blood that affect against the oxidative stress (5). GPX1 gene encode glutathione peroxidase 1 enzyme that plays a role in protecting cells from oxidative stress of which contributes to bone loss by increasing osteoclast activity and reducing osteoblast function. Reduced *GPX1* gene expression or activity may exacerbate oxidative damage, leading to lower BMD and higher fracture risk (6). Studies revealed the role of many miRNAs in bone metabolism through balancing osteoblast and osteoclast cells and their effect on bone disease with high risk of osteoporosis (7-8).The mechanism of these miRNAs in osteoporosis is to promote the osteoclast differentiation, when miRNA levels decrease or increase then effect on the expression of the genes in bones (7-8). The miR-185 gene, located at chromosome22q11.21ncbi.gene/406961 , the precursor composed of 82 nucleotides that produce two mature molecules miR-185-5p and miR-185-3p one located at the 5′ and the other at 3′ at the end of the miRNA precursor molecule respectively Mirbase.mature/MIMAT0000455.

Based on available data, miR-185-5p is considering the predominantly expressed form (9). In their mature form, microRNAs consist of a very short double-stranded RNA molecules, which include a passenger strand that is displaced, and a guide strand that recognizes the target mRNA (10). As shown in figure (1).

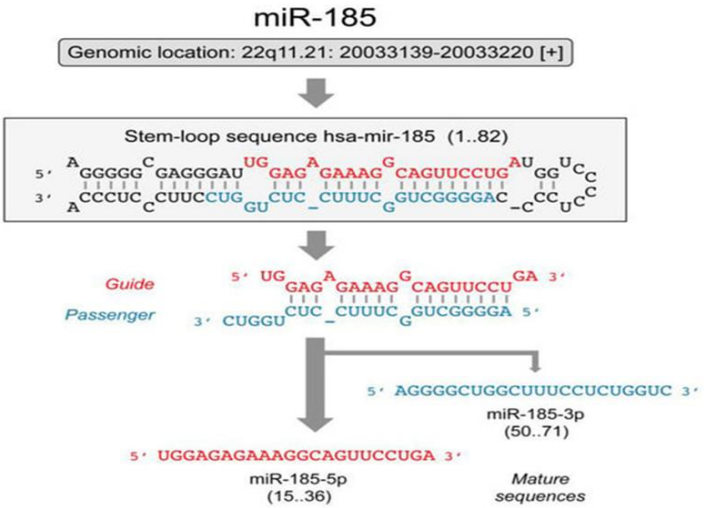

Fig. (1): schematic structure and sequence of miRNA 185, the stem loop structure of precursor and the mature sequence. MiR-185-5p (red labeled strand) and miR-185-3p are two different mature miR-185 sequences (9).

The dysregulation of miRNA-185 (miR-185) has been studied in various human diseases, including metabolic disorders, asthma, frailty, schizophrenia, and hepatitis (11, 12). This study aims to investigate the expression levels of *GPX1* and miRNA 185-5p in Iraqi men with osteoporosis. Additionally, it seeks to evaluate associated biological parameters to identify potential non-invasive diagnostic markers for osteoporosis. The research intends to provide insights into the molecular mechanisms underlying osteoporosis and contribute to the development of improved diagnostic methods in the Iraqi male population.

Materials and methods

Study groups, including and excluding criteria

The study comprise from (30) patient male diagnosed with primary osteoporosis by physicians after Dual-energy X-ray absorptiometry (DEXA) scan examinations and (30) apparently healthy male in age range between (32-75) of which visited Medical city – Baghdad teaching hospital and Alwasity hospital in Baghdad province/Iraq. Both blood samples collection and laboratory practical work were conducted on the groups of study during the period from November 2023 until August 2024. This study was approved by the Ethics Committee of the Department of Biotechnology College of Science, University of Baghdad, Baghdad, Iraq (Ref.: CSEC/1023/0069 on October 30, 2023).as did the Iraqi Ministry of Health and Environment. Prior participation in the study, each participants consented. Specifically excluded individual having the evidence of chronic diseases, systemic and metabolism bone diseases other than primary osteoporosis, none of participant have taken recently any drug that can influence on bone metabolism.

Blood sample collection

The collection of five mL of peripheral blood from each participant was divided into two parts: The first part included (3 ml) of blood samples collected in gel tubes, which were allowed to clot at room temperature for about one hour. Then centrifugation at about 4000 rpm for five minutes was done, the serum was separated into equal parts in Eppendorf tubes. This part was used for serum vitamin D, calcium, and ALP measurements, while the second part included (2 ml) was kept in an EDTA tube. For each tube, added 250 µl of EDTA blood sample to 750 µl of TRIzol reagent (Trans/ India) with pipetting up and down several times to homogenized the lysate. This part used for *GPX1* and miRNA hsa-miR-185-5p gene expression detection. The optimum sterilization conditions for all materials utilized for all experiments in the study were achieved.

Measurement Biochemical parameters

A fully automated analyzer that using an electrochemilinescence binding assay kits by Cobas e 411 (Roche Company, Germany) was used to measure vitamin D in patients and control. While measurements of both serum calcium and alkaline phosphatase enzyme (ALP) were determined by the spectrophotometer Cobas c 111 analyzer system (Roche Company, Germany).

Gene expression of *GPX1*

Determining *GPX1* gene expression involves several techniques to quantify and understand the roles of mRNA and miRNA in biological processes; initiate from total RNA isolation, followed by reverse transcription-quantitative PCR (RT-qPCR) to determine relative mRNA expression levels (13). Total

RNA was isolated following the protocol of *TransZol* Up Plus RNA Kit (TransGen, biotech. ER501-01) Trizo, Trans; India. Using the 2000c Nanodrop spectrophotometer (Thermo Fisher Scientific, USA). To estimate the purity and concentration of the resulted RNA. The process of synthesizing cDNA using the extracted total RNA as template utilizing the *EasyScript*® one step is used for both genomic DNA remover and synthesis first strand of the complementary DNA. Specificdesigned primers which synthesized and lyophilized by Alpha DNA Ltd. (Alpha ADN Montreal, Quebec, Canada). Glyceraldehyde-3-Phosphate Dehydrogenase (*GAPDH*) its NCBI reference sequence is NM_001289746.2. A house keeping gene that considered a crucial factor an endogenous reference for normalization of mRNA level of *GPX1* gene. The amplification of housekeeping genes were also synthesized and lypholyzed and sequence of primers are mentioned in table (1).

The expression levels and fold changes of the *GPX1* and *GAPDH* genes were assessed using *TransStart*® Top Green qPCR Super Mix (TransGen, biotech. AQ131-01)**.** Following the standard protocol, The qRT-PCR reaction was performed in a final volume of 20 µl, consisting of : 10 µl of TransStart® Top Green qPCR SuperMix, 1 µl of each forward and reverse primer (10 µM), 2 µl of cDNA template, and 6 µl of nuclease-free water. The reactions were conducted using the QIAGEN Rotor-Gene Q Real-Time PCR System (Germany) under the following thermal cycling conditions: Initial denaturation: 94°C for 30 sec, Amplification (40 cycles): Denaturation: 94°C for 8 sec, Annealing: 60°C for 15 sec and Extension: 72°C for 20 sec. Finally, Melt curve analysis: 55–95°C (1 cycle). Once a program initiated the software represent data on a spreadsheet or graphical view. Eventually, the relative expression of mRNA findings were presented as a fold change of the *GPX1* gene adjusted to reference *GAPDH* gene that analyzed using the $2^{-\Delta\Delta Ct}$ algorithm and calculating the threshold cycle (Ct) value (13). The CT value of *GPX1* (target gene) has been standardized to that of the control gene. The differences in values of cycle threshold (Ct) between *GAPDH* (the housekeeping gene) and *GPX1* (target gene) has been assed.

Table (1): forward and reverse primers designed and utilized in the study

Primer	Sequence (5′→3′ direction)
GPX1 Forward	CTACGAGGGAGGAACACCTG
Reverse	CTGACACCCGGCACTTTATT
GAPDH Forward	GGCCTCCAAGGAGTAAGACC
Reverse	AGGGGTCTACATGGCAACTG
hsa-miR-185-5p	TGGAGAGAAAGGCAGTTCCTGA
miRU6 F.P.	AGAGAAGATTAGCATGGCCCCT
miRNA-universe R.P.	GCGAGCACAGAATTAATACGAC

miRNA 185 gene expression

Total miRNA was isolated from all samples using the EasyPure® miRNA Kit (TransGen Biotech, Catalog No. ER601-01) following the manufacturer's protocol. The expression levels of miR-185-5p and the endogenous reference gene U6 (NCBI RefSeq: NR_004394) were quantified using SYBR Green-based reverse transcription-quantitative PCR (RT-qPCR) using the TransStart® Top

Green qPCR SuperMix (TransGen Biotech, AQ131-01). The reaction setup in total volume: 20 µL consisting of: 10 µL TransStart® Top Green qPCR SuperMix, 1 µL each of forward and reverse primers (10 µM) (Table 1), 2 µL cDNA template, 6 µL nuclease-free water. Thermal Cycling Conditions (Rotor-Gene Q System, QIAGEN, Germany). Initial denaturation: 94°C for 30 sec, Amplification (40 cycles): Denaturation: 94°C for 10 sec, Annealing: 60°C for 15 sec, Extension: 72°C for 20 sec. Melt curve analysis: 55°C to 95°C (1 cycle). Gene expression was normalized to U6, the threshold cycle (Ct) values were determined and relative quantification was performed using the $2^{-\Delta\Delta Ct}$ method (14). Fold changes were calculated by comparing target gene expression in experimental samples to that of the healthy control calibrator.

Statistical analyses

Study results were subjected to a statistical evaluation with IBM SPSS Statistics 27.0 (Armonk, NY: IBM Corp.). Graphs were plotted with Graph-Pad Prism 10.4.1(San Diego, California USA). Categorical variables were expressed using numbers and percentages. The results reported in this study were expressed as mean ± SD. Independent t-test was used to test between two parameters. One-way ANOVA were used to test between study groups. Probability values less than 0.05 and 0.01 were considered significantly and highly significant different, respectively.

Result and discussion

Demographic and laboratory parameters

All participants in the study were of Iraqi ethnicity that showed no significant differences in demographic factors between primary osteoporosis patients and healthy controls. The patient's group included 30 men with primary osteoporosis (Mean±SD 58.57±9.310 years) while the control group consisting of 30 healthy individuals, was age-matched to the osteoporosis group (Mean±SD 60.23±8.768 years) and the p value= 0.478 that not differ significantly across the study groups. Also the BMI indicates no significant difference between osteoporosis patients and healthy group (Mean±SD 28.70 ± 3.865) and (Mean±SD29.02 ± 4.272) respectively with p value= 0.762. Meanwhile as presented in figure (2), the BMD and T-score in the osteoporosis group (Mean±SD 0.755 ± 0.033g/cm²) and (Mean±SD -2.857 ± 0.260) respectively when compared to the control group (Mean±SD 0.884 ± 0.035 g/cm²) and (Mean±SD 0.156 ± 0.666) respectively, showed a significant decrease with a p-value of <0.0001. This is consistent with the diagnostic criteria for osteoporosis, which is defined by a BMD together with T-score significantly lower than the reference population. The substantial difference in BMD highlights the severity of bone loss in the osteoporosis group. These findings align with De Martinis with his colleagues and Vilaca with his colleagues which found that BMD and mean T score values were significantly decrease in osteoporosis men (15-16). ALP results showed elevation among osteoporosis patients (Mean±SD182.0 ± 32.73 U/l) against healthy control (Mean±SD109.4 ± 45.08 U/l) the significant differences *P* value was less than <0.0001. Elevated ALP levels are a marker of increased bone turnover, which is characteristic of osteoporosis. These results in agreement with Choi and his colleagues that reported high correlate with bone resorption markers and fracture risk in postmenopausal

women and older men (17). In contrast, serum calcium decreased in osteoporosis patients (Mean±SD 7.010 ±1.719 mg/dl) while in healthy control was (Mean±SD 9.130 ± 0.985 mg/dl) with (*P* value <0.0001). The vitamin D levels were low in osteoporosis men in comparison with healthy control (Mean±SD 18.30 ± 5.995 ng/ml and Mean±SD 23.10 ± 6.099 ng/ml) respectively. With significant p value (=0.0023 **).

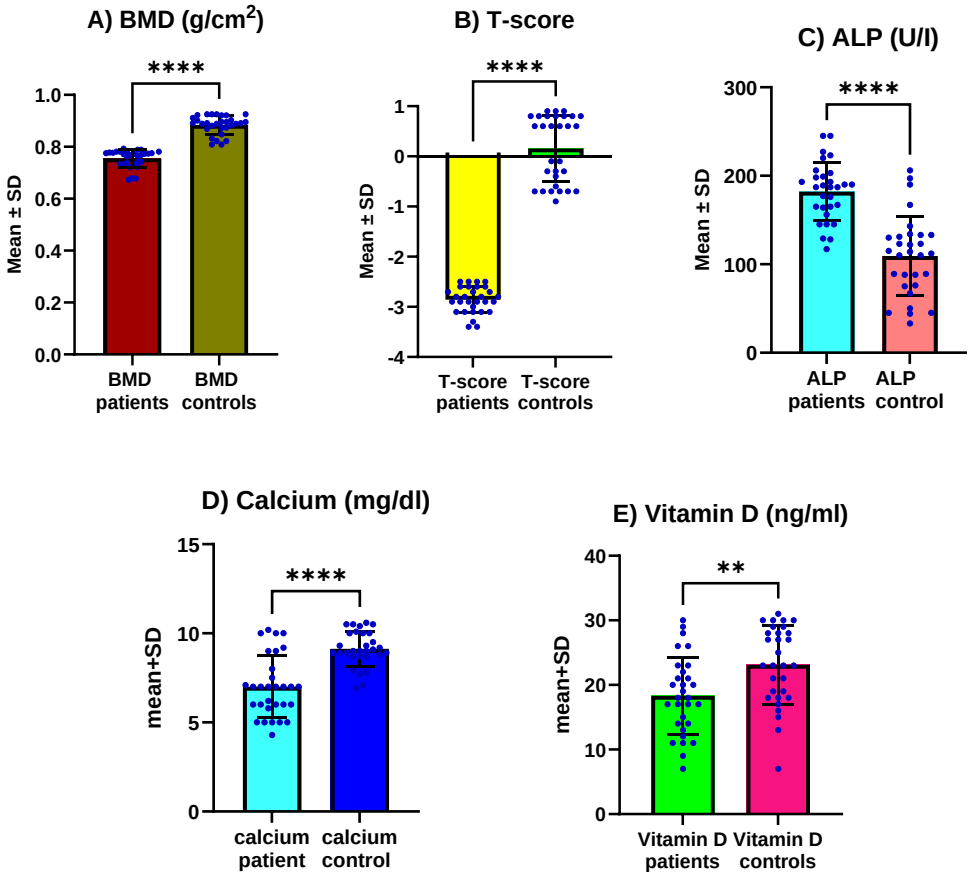

Fig. (2): Column- bar plot of A)BMD; B) T-score; C) APL ; D) calcium and E) Vitamin D; in men with osteoporosis disease and healthy control (HC). Column represent mean. Bar represent standard deviation (SD). Mann- Whitney test used to assess significant differences (*p> 0.05), (**p> 0.0001) and non- significant differences (p<0.05): ns.**

Calcium is a critical component of bone matrix, and hypocalcemia can exacerbate bone loss. The lower calcium levels in the osteoporosis group may reflect inadequate dietary intake, poor absorption due to vitamin D deficiency, or increased calcium excretion. Al-Juaifari(2024) and Shlisky(2022)(18-19). Vitamin D deficiency in human serum determine a significant risk factor for osteoporosis, particularly in older adults (16,18). These results demonstrate significant differences in BMD, T-scores, vitamin D, ALP, and calcium levels between men with osteoporosis and the control group which spot the light on the multifactorial osteoporosis in men as a significant public heath, emphasizing the importance of early screening for BMD, vitamin D, and calcium levels in at-risk populations.

Molecular Analysis

This study investigated miRNA expression in osteoporosis patients, as miRNAs have the potential to serve as

biomarkers influencing post-transcriptional gene regulation. Successful extraction of total RNA from all blood samples of study groups done by *TransZol* Up Plus RNA Kit. The range of RNA concentration was (low: 4.6 ng/µl to high: 44.1 ng/µl). No significant differences was observed in concentration of total RNA between osteoporosis patients and healthy control samples. Similarly, RNA purity was ranged from (low: 1.88 ng/µl to high: 2.02 ng/µl) has shown no significant differences among the groups. Both high concentration and yield of total RNA mostly depend on the strictly aseptic extraction conditions through TranZol up kit reagents that widely reported (20).

GPX1 and _miRNA 185-5p_ genes Expression:

Expression levels of *GPX1* and *miRNA185-5p* were analyzed by RT-qPCR using *GAPDH* and *U6* as endogenous controls, respectively, with quantification via the $2^{-\Delta\Delta Ct}$ method the fold change in patients is equal $2^{-\Delta\Delta Ct}$. The *GPX1* gene exhibited a modest decrease in the mean value of the $2^{-\Delta\Delta Ct}$ of osteoporotic men (mean ±SD 0.886±0.48) compared to healthy controls (mean ± SD1.0±0.26) with no significant difference. As shown in table (3). The amplification profile for *GPX1* is presented in figure (3).

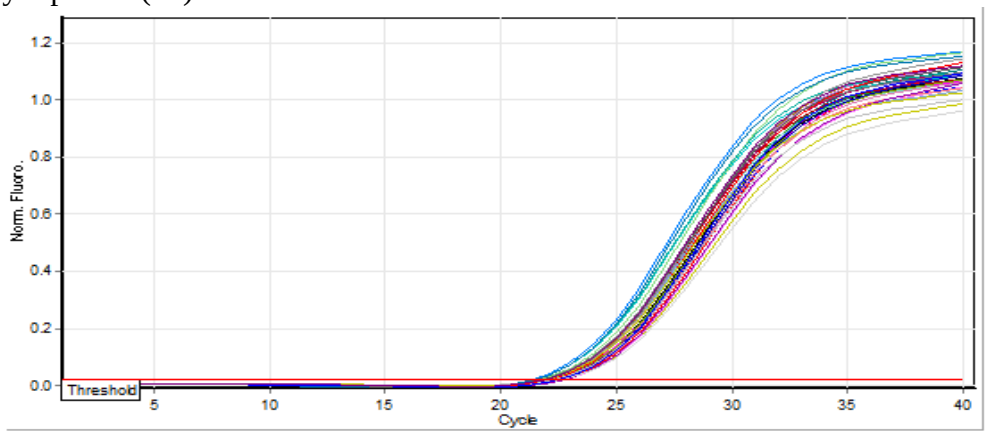

Fig. (3): Real-time PCR amplification plot of _GPX1_ gene expression. The plot depicts fluorescence intensity versus cycle number for _GPX1_ amplification across all study groups, as measured by the Rotor-Gene Q real-time PCR system (Qiagen).

Table (3): The _GPX1_ expression fold in osteoporosis men and healthy control

Groups	*Means of GPX1 CT*	*Means of GAPDH CT*	*ΔCT*	*Δ ΔCT*	$2^{-\Delta\Delta Ct}$
control	22.02±0.45	13.83±0.57	8.18±0.17	0.00±0.82	1.0±0.26
Patients	22.36±0.26	13.89± 0.32	8.46± 0.3	0.16±0.17	0.88±0.6

NS=Non Significant, ΔCT = Delta Cycle Threshold.

Similarly, *miRNA185-5p* displayed a slight but non-significant upregulation in men with osteoporosis (mean ± SD 1.2±0.14) while the control group (mean ± SD 1.0±0.4). The amplification plot for the *miRN185 -5p* gene is depicted in figure (4). Table (4) show the gene expression of the *miRNA185-5p*

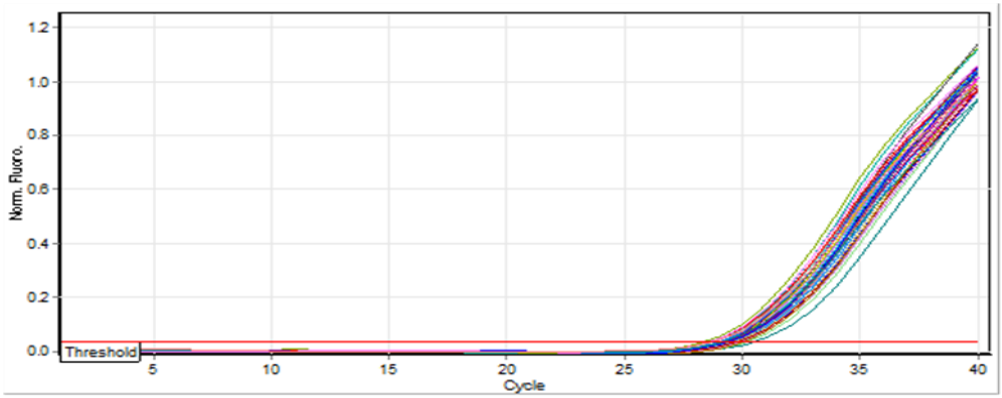

Fig. (4): Real-time PCR amplification plot of *miRNA 185-5p* gene expression. The plot depicts fluorescence intensity versus cycle number for *miRNA185-5p* amplification across all study groups, as measured by the Rotor-Gene Q real-time PCR system (Qiagen).

Table (4): The *miRNA185-5p* expression fold in osteoporosis men and healthy control

Groups	***Means of* miRNA 185-5p *CT***	***Means of* U6 *CT***	***ΔCT***	***Δ ΔCT***	$2^{-\Delta\Delta Ct}$
Control	29.0±0.7	23.46±0.14	5.53±0.62	0.00±0.62	1.0±0.4
Patients	29.36±0.65	24.02±0.78	5.34±0.13	0.19±0.13	1.2±0.14

The slight downregulation of *GPX1* in osteoporosis men may indicate a compensatory response to oxidative stress in osteoporosis, of which may lead to exhaustion of antioxidant defenses including glutathione peroxidase, due to persistent reactive oxygen species (ROS) accumulation. High ROS levels might suppress *GPX1* expression as part of a dysregulated redox balance. In addition to sex factor, Men may have different oxidative stress responses compared to women making them more susceptible to bone loss under oxidative conditions (21-22). Whereas the upregulation of *miR-185* could reflect its role in bone metabolism that interacted with oxidative stress and may drive bone loss by disrupting osteoblast-osteoclast equilibrium (22-23). Consistent with previous studies; *miR-185-5p* downregulates *GPX1* to suppress AML progression (21). Another study reported that *miR-185-5p* inhibits osteogenesis in bone cells (22). Micro RNAs have a role in development and diagnosing primary osteoporosis disease. Hence various miRNAs were studied to determine their roles in osteoporosis mentioned their different expression and have a potential to serve as biomarkers for osteoporosis diagnosis and progression monitoring but require more experimental studies on large population with taking advantage of age and sex factors (23). An increasing amount of evidence highlights the pivotal role of microRNAs in gene regulation of bone formation and resorption, ensuring bone homeostasis. However, under pathological conditions, dysregulated miRNA signaling contributes to skeletal disorders like osteoporosis (24-25). MiR-185 helps in maintaining bone health by regulating processes like bone formation, cell growth, and fracture healing. It works through key numerous pathways but when its levels are imbalanced, it can disrupt

bone development or contribute to conditions like osteoporosis. These insights suggest miR-185 could be a promising target for treating bone-related diseases and improving recovery from fractures (26-27). Hence antioxidants are important against oxidative stress that cause many pathological conditions Hussein (2024) and Khalid (2024) (28-29). This suggests that oxidative stress plays a key role in the pathophysiology of osteoporosis in men, and enhancing antioxidant defenses through *GPX1* activation could be a promising therapeutic strategy (26-27). Osteoporosis in men is frequently underdiagnosed due to limited awareness of risk factors like aging, hormonal imbalances, and lifestyle habits, Diagnosis relies on BMD assessment, DXA scan, yet inadequate treatment persists, impacting quality of life (30-31). Studies emphasized the importance of sex-specific approaches in osteoporosis management. Highlighting the distinct pathophysiological mechanisms in male osteoporosis, particularly the role of oxidative stress and reduced antioxidant defense (31-32). These findings underscore the need for strategies that address the unique metabolic and genetic factors contributing to bone loss in men. Future research should target both bone resorption and oxidative stress pathways to optimize outcomes in male osteoporosis which is important in diagnosis and as therapeutic management.

Conclusion

Osteoporosis is becoming more common among men due to as life expectancy increases, making it a growing health concern. This emphasize the importance of raising awareness concerning disease risk factors and the potential role of miRNAs and oxidative stress in its diagnosis and management. By promoting early detection, to overcome the impact of osteoporosis on men's lives. Further research and targeted public health efforts are required.

References

1. Noel, S.E.; Santos, M.P. and Wright, N.C.; (2020). Racial and ethnic disparities in bone health and outcomes in the United States. *Journal of Bone and Mineral Research*, 36(10), pp.1881-1905.
2. Onizuka, N. and Onizuka, T.; (2024). Disparities in osteoporosis prevention and care: understanding gender, racial, and ethnic dynamics. *Current Reviews in Musculoskeletal Medicine*, *17*(9), pp.365-372.
3. Aibar-Almazán, A.; Voltes Martínez, A.; Castellote Caballero, Y.; Afanador Restrepo, D.F.; Carcelén-Fraile, M.D.C. and López Ruiz, E.; (2022). Current Status of the Diagnosis andManagement of Osteoporosis.
4. Sun, C.; Qi, B.; Huang, X.; Chen, M.; Jin, Z.; Zhang, Y.; Zhu, L. and Wei, X.; (2022). Baduanjin exercise: a potential promising therapy toward osteoporosis. *Frontiers in Medicine*, *9*, p.935961.
5. Handy, D.E. and Loscalzo, J.; (2022). The role of glutathione peroxidase-1 in health and disease. *Free Radical Biology and Medicine*, *188*, pp.146-161.
6. Liu, C.; Yan, Q.; Gao, C.; Lin, L. and Wei, J.; (2021). Study on antioxidant effect of recombinant glutathione peroxidase 1. *International Journal of Biological Macromolecules*, *170*, pp.503-513.
7. Zhao, S.L.; Wen, Z.X.; Mo, X.Y.; Zhang, X.Y.; Li, H.N.; Cheung, W.H.; *et al.* (2022). Bone-metabolism-related serum microRNAs to diagnose osteoporosis in middle-aged and elderly women. *Diagnostics*, *12*(11), p.2872.
8. Yang, Y.; Yujiao, W.; Fang, W.; Linhui, Y.; Ziqi, G.; Zhichen, W.; *et al.* (2020). The roles of miRNA, lncRNA and circRNA in the development of osteoporosis. *Biological research*, *53*.

9. Babaeenezhad, E.; Naghibalhossaini, F.; Rajabibazl, M.; Jangravi, Z.; Hadipour Moradi, F.; Fattahi, M.D.; *et al.* (2022). The roles of microRNA miR-185 in digestive tract cancers. *Non-coding RNA*, *8*(5), p.67.
10. Diener, C.; Keller, A. and Meese, E.; (2024). The miRNA–target interactions: an underestimated intricacy. *Nucleic Acids Research*, *52*(4), pp.1544-1557.
11. Condrat, C.E.; Thompson, D.C.; Barbu, M.G.; Bugnar, O.L.; Boboc, A.; Cretoiu, D., *et al.* (2020). miRNAs as biomarkers in disease: latest findings regarding their role in diagnosis and prognosis. *Cells*, *9*(2), p.276.
12. Pordel, S.; Khorrami, M.; Saadatpour, F.; Rezaee, D.; Cho, W.C.; Jahani, S.; *et al.* (2023). The role of microRNA-185 in the pathogenesis of human diseases: a focus on cancer. *Pathology-Research and Practice*, *249*, 154729.
13. Dawood, H.H. and Mohammed, R.K.; (2023). The Correlation Study between TP53 Gene Expression and Acute Myeloid Leukemia in Iraq. *Iraqi Journal of Science*, pp.5615-5623.
14. Kuang, J.; Yan, X.; Genders, A.J.; Granata, C. and Bishop, D.J. (2018). An overview of technical considerations when using quantitative real-time PCR analysis of gene expression in human exercise research. *PloS one*, *13*(5), p.e0196438.
15. De Martinis, M.; Sirufo, M.M.; Polsinelli, M.; Placidi, G.; Di Silvestre, D. and Ginaldi, L. (2020). Gender differences in osteoporosis: a single-center observational study. *The world journal of men's health*, *39*(4), p.750.
16. Vilaca, T.; Eastell, R. and Schini, M.; (2022). Osteoporosis in men. *The lancet Diabetes and endocrinology*, *10*(4), pp.273-283.
17. Choi, K.H.; Lee, J.H. and Lee, D.G.; (2021). Sex-related differences in bone metabolism in osteoporosis observational study. *Medicine*, *100*(21), p.e26153.
18. Al-Juaifari, B.A.J.; Al-Jumaili, E.F.A. and Mahdi, A.A.; (2024). The role of *Parathyroid Hormone Receptor 1* gene polymorphism at rs1138518 SNP in the incidence of osteoporosis in a sample of Iraqi women. *Iraqi Journal of Biotechnology*, 23(2).
19. Shlisky, J.; Mandlik, R.; Askari, S.; Abrams, S.; Belizan, J.M.; Bourassa, M.W.; *et al.* (2022). *Calcium deficiency worldwide: prevalence of inadequate intakes and associated health outcomes*, 1512(1): 10-28.
20. Kamel, M.D. and Kandala, N.J. (2023). The Potential Regulatory Role of miR-378 in the Expression of Bone Morphogenetic Protein-15 in Infertile Women with Hyperprolactinemia (HPL). *Iraqi Journal of Science*, pp.5580-5590.
21. Pang, B.; Mao, H.; Wang, J. and Yang, W. (2022). MiR-185-5p suppresses acute myeloid leukemia by inhibiting GPX1. *Microvascular Research*, *140*, p.104296.
22. Sun, D.; Liu, J.; Shi, Q.; Mu, H. and Zhou, D. (2018). Regulatory role of microRNA-185 in the recovery process after ankle fracture. *Experimental and Therapeutic Medicine*, *16*(4), pp.3261-3267.
23. Jiménez-Ortega, R.F.; Ortega-Meléndez, A.I.; Patiño, N.; Rivera-Paredez, B.; Hidalgo-Bravo, A. and Velázquez-Cruz, R. (2024). The involvement of microRNAs in Bone Remodeling Signaling pathways and their role in the development of osteoporosis. *Biology*, *13*(7), p.505.
24. Panni, S. and Pizzolotto, R.; 2025. Integrated Analysis of microRNA Targets Reveals New Insights into Transcriptional–Post-Transcriptional Regulatory Cross-Talk. *Biology*, *14*(1), p.43.
25. Bellavia, D.; De Luca, A.; Carina, V.; Costa, V.; Raimondi, L.; Salamanna, F.; *et al.* (2019). Deregulated miRNAs in bone health: Epigenetic roles in osteoporosis. *Bone*, *122*, pp.52-75.
26. Lu, C.; Zhou, D.; Wang, Q.; Liu, W.; Yu, F.; Wu, F. and Chen, C.; (2020). Crosstalk of MicroRNAs and oxidative stress in the pathogenesis of cancer. *Oxidative Medicine and Cellular Longevity*, *2020*(1), p.2415324.
27. Trojniak, J.; Sendera, A.; Banaś-Ząbczyk, A. and Kopańska, M.; (2024). The MicroRNAs in the Pathophysiology of Osteoporosis. *International Journal of Molecular Sciences*, *25*(11), p.6240.
28. Hussein, Z.T. and Abdul-Hassan, I.A.; (2024). The association of Paraoxonase 1 (PON-1) gene polymorphism at rs854560 SNP (L55M) and some oxidative stress parameters in serum with the incidence of recurrent

spontaneous abortion in Iraqi women. *Iraqi Journal of Biotechnology*, 23(2): 185–192.

29. Khalid, M.M. (2024). Variants in the Antioxidant Gene (catalase) and its Correlation with Idiopathic Male Infertility in Iraqi Population. *Iraqi journal of biotechnology*, *23*(1).
30. Vescini, F.; Chiodini, I.; Falchetti, A.; Palermo, A.; Salcuni, A.S.; Bonadonna, S., *et al*. (2021). Management of osteoporosis in men: a narrative review. *International Journal of Molecular Sciences*, *22*(24), 13640.
31. Jeong, C.; Ha, J.; Kim, J.; Lim, Y.; Kim, M.K.; Kwon, H.S., *et al.* (2022). The efficacy of denosumab in Korean male patients with osteoporosis. *The Korean journal of internal medicine*, *37*(5): 1011.
32. Bandeira, L.; Silva, B.C. and Bilezikian, J.P. (2022). Male osteoporosis. *Archives of endocrinology and metabolism*, *66*(5), pp.739-747.