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ADVANCES IN THE DIAGNOSIS AND MOLECULAR UNDERSTANDING OF OSTEOPOROSIS: EMPHASIS ON PROTEOMICS AND BIOMARKER DISCOVERY

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ABSTRACT
Osteoporosis and osteopenia are major metabolic bone disorders characterized by reduced bone mineral density (BMD) and deterioration of bone microarchitecture, ultimately increasing fracture risk. Despite the availability of multiple diagnostic modalities, early detection remains challenging due to methodological limitations and biological variability. This review provides a comprehensive overview of bone biology, epidemiology, molecular signaling pathways, diagnostic strategies, and therapeutic approaches. Special emphasis is placed on proteomics and its emerging role in biomarker discovery. Recent advances in omics technologies have enabled high-throughput molecular profiling, offering deeper insights into disease mechanisms and paving the way for personalized medicine. The integration of proteomics into clinical practice holds promise for improving early diagnosis, prognosis, and targeted therapy in osteoporosis.

KEYWORDS: Osteoporosis, Dual-energy x-ray absorptiometry (DEXA), Bone mineral density (BMD), Proteomics, Bone.

1. INTRODUCTION
Bone is a dynamic and metabolically active connective tissue composed of a highly organized matrix and specialized cell types, including osteoprogenitor cells, osteoblasts, osteoclasts, and osteocytes [1]. These cells collectively regulate bone formation, maintenance, and resorption. Bone remodeling is a continuous physiological process in which old or damaged bone is replaced by new bone tissue through tightly coupled phases of resorption and formation [2]. This process is essential for maintaining skeletal strength and mineral homeostasis. Bone turnover represents the proportion of bone mass replaced over a defined period and is critical for understanding skeletal health [3]. Remodelling of bone is considered a physiological process resulting from ongoing cellular processes. Earlier models described bone remodeling as a simple interaction between osteoblasts and osteoclasts; however, recent advances have revealed a complex network involving multiple signaling pathways, cellular interactions, and mechanical stimuli [4,5]. These processes ensure adaptation to physiological demands and preservation of bone integrity. Metabolic bone diseases, including osteoporosis, osteomalacia, and rickets, represent a significant health burden worldwide [6,7]. Among these, osteoporosis is the most prevalent and is associated with increased morbidity and mortality due to fractures [8].
Osteoporosis is broadly classified into primary and secondary types [9]. Primary osteoporosis includes postmenopausal and age-related fo rms, while secondary osteoporosis results from underlying medical conditions, medications, or lifestyle factors. The disease is often asymptomatic until fractures occur, most commonly affecting the hip, spine, and wrist [10]. Osteoporosis can be detected with the help of bone densitometry as it leads to deterioration of the microarchitecture of the bone [11,12]. Low bone mass, which is osteopenia, is not considered a disease category but is intended only for epidemiological description. Osteopenia, if untreated, can lead to osteoporosis [13]. The t-score values in a dual-energy x-ray absorptiometry (DEXA) scan can differentiate these conditions. Osteopenic patients become more prone to fractures. Histological samples show that the trabeculae are much thinner, the osteon size is smaller, and the Haversian and bone marrow spaces are larger [14]. Global prevalence estimates suggest that osteoporosis affects a substantial proportion of the population, with a higher burden observed in women, particularly after menopause [15]. Regional variations exist, with higher prevalence in developing countries and Asian populations [16,17]. In India, the burden is significant due to nutritional deficiencies, lack of awareness, and limited access to diagnostic facilities [19,20]. Key risk factors include advancing age, hormonal imbalance (especially estrogen deficiency), inadequate calcium and vitamin D intake, sedentary lifestyle, smoking, alcohol consumption, and genetic predisposition (Figure 1) [20,21].

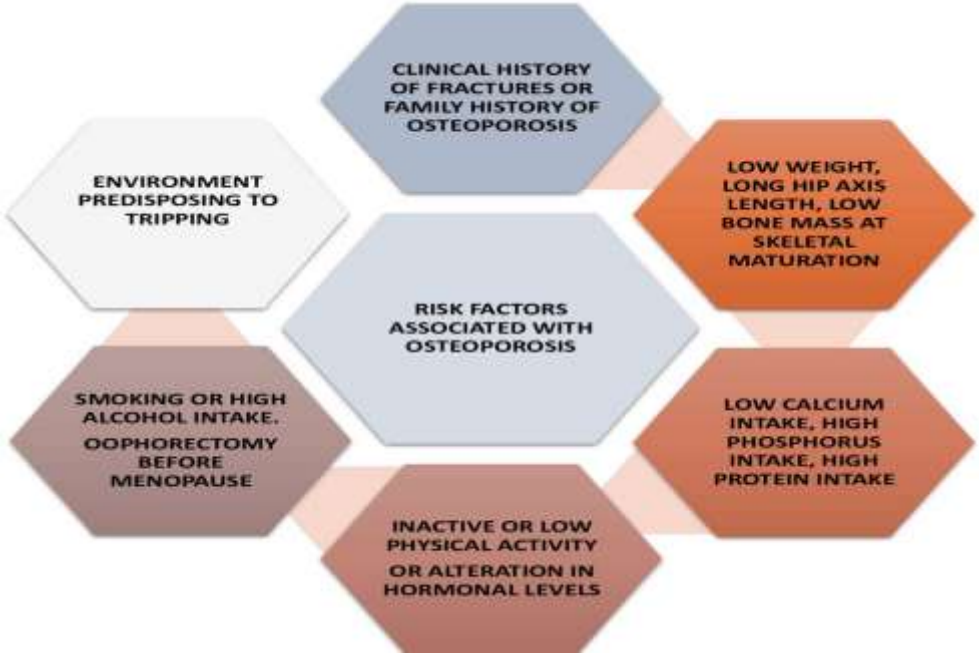

Figure 1: Illustration of major risk factors contributing to osteoporosis, including lifestyle, dietary, hormonal, and genetic factors.

2. Molecular Mechanisms of Bone Remodeling

Bone remodeling is regulated by multiple signaling pathways that coordinate the activity of osteoblasts and osteoclasts.

2.1 RANK/RANKL/OPG Pathway

The RANK/RANKL/OPG signaling pathway plays a central role in osteoclast differentiation and activation. RANK is a member of the tumour necrosis factor (TNF) family, but unlike other TNF family receptors, RANK lacks any innate protein kinase activities that help regulate the signalling pathway. TRAFs 2, 5, and 6 are associated with RANK, but only TRAF 6 is responsible for bone health. Several studies depict that a deficit in TRAF6 leads to the development of osteoporosis. RANKL binds to its receptor RANK on osteoclast precursors, promoting their maturation and bone-resorbing activity. Osteoprotegerin acts as a decoy receptor, inhibiting this interaction and thereby reducing bone resorption (Figure 2). An imbalance in this pathway leads to increased osteoclast activity and bone loss [22–24].

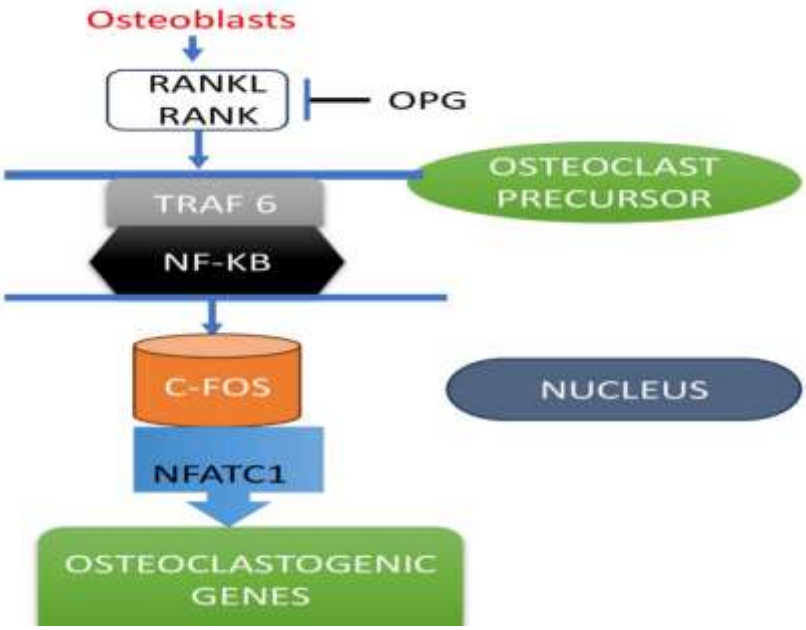

Figure 2: Representation of the RANK–RANKL–OPG pathway highlighting osteoclast activation and regulatory mechanisms.

2.2 Wnt/β-Catenin Signaling

The Wnt signaling pathway is crucial for osteoblast differentiation and bone formation [25–27]. Activation of β-catenin promotes gene transcription involved in osteogenesis, while inhibitors such as sclerostin suppress this pathway. β-catenin is not phosphorylated in the presence of a Wnt ligand, and it aggregates in the nucleus. This pathway regulates the

downstream target genes of Wnt. FZD and LRP5/6 receptors are prominently required in the Wnt, or β-catenin, signalling pathway. Dysregulation of Wnt signaling is strongly associated with reduced bone formation and osteoporosis [28,29].

2.3 Notch Signaling

Notch signaling regulates both osteoblast and osteoclast function. Notch signals are mostly juxtacrine. This pathway plays a dimorphic role in bone turnover. It exhibits a dual role by influencing bone formation and resorption depending on the cellular context. Its interaction with other pathways underscores its importance in maintaining bone homeostasis. Notch 1-4 receptors are known to play different roles in bone tissue. Osteoclast differentiation is suppressed by Notch1 signals. When forming osteoclasts, RANKL is believed to trigger the production of Notch 2, thereby promoting the creation of osteoclasts. Researchers reported in vitro experiments that the deletion of Notch 3 in bone marrow macrophages enhances osteoclast genesis. Notch 4 is upregulated during osteogenic differentiation [30–35]. Osteoporosis is clinically characterized by fragility fractures resulting from minimal trauma [36]. The most common sites include the hip, vertebrae, and wrist. Estrogen deficiency and aging are the primary contributors to primary osteoporosis, whereas secondary osteoporosis arises due to systemic diseases, medications, or lifestyle factors [37–39].

3. Diagnostic Approaches

3.1 Imaging Techniques

Several biochemical and radiological techniques are known that can help in the diagnosis of low bone mineral density. Techniques commonly used for diagnosis include quantitative computed tomography (QCT), ultrasounds, dual-energy X-ray absorptiometry (DEXA) scans, X-rays, and magnetic resonance imaging (MRI) procedures [40]. Dual-energy X-ray absorptiometry (DEXA) is the gold standard for measuring BMD and classifying osteoporosis using T-scores [41]. Radiographs can also assist in estimating BMD. Singh et al. described a simple, semi-quantitative evaluation tool in 1970, which is currently known as the Singh Index (SI) [42]. According to the SI, osteoporosis can be classified into six grades from 1 to 6 (Table *1*).

Table 1: Singh Index Classification

Grading	Bone deformity	Interpretation
Grade V and VI	normal to near normal	Grade VI. Trabecular bone groups are completely visible. Cancellous bone fills the upper femur end. Fine strands of trabecular bone are visible in the Ward's triangle. Grade V. Ward's Triangle, primary compressive, and tensile trabeculae are observable. The secondary groups of trabeculae are less clearly visible.
Grade III and IV	Osteopenia	Grade IV. Secondary compressive trabeculae were reduced to one-inch tensile trabeculae. Resorption radially spreads from the center. Grade III. The primary compression trabeculae were seen, with a reduction of one tensile trabecula. They are only visible in the upper part of the neck of the femur but can no longer be traced to the greater trochanter.
Grade I and II	Osteoporosis	Grade II. One-inch tensile trabeculae are not seen, but the compressive group is visible. Grade I. Minimal compressive trabeculae.

Table 1: Grades used for decreased bone mineral density.

The Singh Index provides a semi-quantitative method for assessing bone loss based on trabecular patterns in radiographs. Higher grades indicate normal bone, while lower grades indicate severe osteoporosis.

DEXA is interpreted in terms of t-scores. T-score greater than or equal to -1.0: Normal, less than -1.0 to greater than -2.5: osteopenia, less than or equal to -2.5: osteoporosis, less than or equal to -2.5 plus fragility fracture: severe osteoporosis [45]. Z-scores, however, remain an important concept because they can be used to express the risk of the patient sustaining an osteoporotic fracture as compared to his age-matched peers [46].

3.2 Biochemical Markers

Biochemical markers of bone turnover provide insights into bone metabolism [47]. These include markers of bone formation such as osteocalcin and bone-specific alkaline phosphatase, and markers of bone resorption such as CTX and NTX. These markers are useful for monitoring disease progression and treatment response (Table 2).

Marker	Description/Significance
Osteocalcin	Present in many immunoreactive forms in the blood, mainly produced by osteoblasts.
Bone-specific alkaline phosphatase	Produced specifically by the osteoblasts.
P1NP- (N-amino terminal polypeptide)	Derived specifically from proliferating osteoblasts and fibroblasts.
P1CP- (C- carboxy-terminal polypeptide)	Produced specifically by the proliferating osteoblasts and fibroblasts.
Bone sialoproteins	Synthesized by osteoblasts and osteoclast-like cells, in bone extra-cellular matrix. It is a phosphorylated glycoprotein.

Urinary calcium	High levels are useful in the detection of high turnover osteoporosis.
Tartrate-resistant acid phosphatase	Six isoenzymes are present in humans. TRAP is synthesized by osteoclasts, macrophages, dendritic cells, etc. It plays a role in the development of skeleton, formation, and degradation of collagen, the calcification of bone, cytokine production by macrophages and dendritic cells, and recruitment of macrophages and dendritic cells. TRAP can destroy skeletal phosphoproteins.
Urinary hydroxyproline	Found mainly in every fibrillar collagen and partly collagenous proteins example elastin and also seen in recently synthesized and mature collagen.
Cathepsin K & L	Cathepsin K, a cysteine protease, has an important role in osteoclast-mediated bone matrix degradation. It cleaves the helical and telopeptide regions of collagen type 1. Cathepsin K & L activate the latent enzyme TRAP. Cathepsin L plays a similar function in macrophages.
PYD & DPD (Pyridinamines & deoxypyridinolines)	These are present in mature collagens only. Elevated in patients with metabolic bone diseases.
C& N terminal telopeptides (CTX & NTX)	Both are fragments of type 1 collagen from the telopeptide region. CTX is a specific product of cathepsin K-mediated bone resorption.
DKK-1 (Dickkopf related proteins -1) SOST (Sclerostin)	Markers are still under research

Table 2: Bone Turnover Markers

A wide range of markers reflect bone formation and resorption processes, including osteocalcin, P1NP, TRAP, and collagen degradation products.

4. Limitations

Despite advancements, diagnostic tools have limitations including high cost, limited accessibility, variability in results, and inability to detect early-stage disease accurately. DEXA scan of the spine (PA) doesn't provide a clear measurement of trabecular bone density because it can be affected by cortical bone, spinous processes, and trabecular bone, and it might not always detect or exclude degenerative changes in the spine. Quantitative ultrasound (QUS) is a non-invasive and feasible procedure, but is not considered the gold standard for the diagnosis of osteoporosis. In Quantitative Computed Tomography (qCT), both the cortical and spongy bone can be examined separately. But it has some disadvantages too. Firstly, it does not provide any estimation of bone mineral content, and secondly, the radiation dose is very high. The technique is very costly and also requires trained technicians. X-rays are a cheaper alternative, but they can detect osteoporosis only after a significant loss of bone mass [48–51].

5. Therapeutic Strategies

Management of osteoporosis includes lifestyle modification, nutritional supplementation, and pharmacological treatment. Calcium and vitamin D supplementation form the basis of therapy. Vitamin D3 helps in the calcification of bones and the reabsorption of calcium from kidney tubules. Pharmacological options include anti-resorptive agents such as bisphosphonates and denosumab, and anabolic agents such as teriparatide and romosozumab. Denosumab is a type of monoclonal antibody, and it inhibits RANKL, which works by decreasing the development of osteoclasts. Bisphosphonates inhibit the actions of osteoclasts. Anabolic agents like teriparatide, abaloparatide, and romosozumab are also available. Anabolic agents should be prescribed to patients with severe OP or high fracture risk. Teriparatide is a fragment of the human parathyroid hormone (PTH). In contrast to pulsed doses, which cause bone formation, PTH typically causes bone resorption. Physicians use Abaloparatide, an analogue of the parathyroid hormone-related protein (PTHrP), to treat osteoporosis. Romosozumab (Evenity®) is a man-made antibody that helps build bone and stops bone breakdown by blocking a protein called sclerostin, which helps control how bones are formed [52–56]. Hormone replacement therapy may be considered in postmenopausal women [57].

6. Omics Technologies in Osteoporosis

Omics technologies enable comprehensive analysis of biological systems at multiple levels, including genes, transcripts, proteins, and metabolites. These technologies can be applied to a biological system of interest to obtain a snapshot of underlying biology at a very high resolution. The interdisciplinary nature of omics is revealed as it aggregates biology and bioinformatics to scrutinise the relationships and interplay between proteins and other molecules, and helps to find the importance of those interactions. Many specialized repositories have been established to curate and disseminate data generated from high-throughput omics investigations. Prominent examples include The Cancer Genome Atlas (TCGA), the International Cancer Genome Consortium (ICGC), the ProteomeXchange Consortium, the Proteomics Identifications Database (PRIDE), and the Human Metabolome Database (HMDB). These platforms serve as publicly accessible repositories that enhance transparency, reproducibility, and data sharing in scientific research, thereby strengthening the evidentiary basis of published studies. The overarching objective of omics technologies is the comprehensive and systematic characterization of biological molecules within a given system. This includes the large-scale analysis of genes (genomics), messenger RNA transcripts (transcriptomics), proteins (proteomics), and small-molecule metabolites

(metabolomics) derived from biological samples. Collectively, these four domains—genomics, transcriptomics, proteomics, and metabolomics—are commonly referred to as the "four major omics," reflecting their central role in advancing systems-level understanding of biological processes and disease mechanisms [58–63]. These approaches have significantly enhanced our understanding of disease mechanisms and biomarker discovery. Advancements in microarray-based platforms have significantly accelerated research in genomics and transcriptomics by enabling high-throughput analysis of gene expression patterns. In parallel, analytical techniques such as mass spectrometry coupled with liquid chromatography have become central to proteomic and metabolomic investigations, facilitating the sensitive detection and precise identification of diverse biomolecules [64].

7. Proteomics and Biomarker Discovery

Proteomics involves large-scale analysis of proteins, which are the functional molecules in biological systems. Omics-based approaches play a critical role in biomarker discovery, particularly in understanding disease onset, progression, and therapeutic response. This capability arises from the integrated characterization and quantification of molecular components across biological systems, allowing for a systems-level perspective of pathophysiological processes. The emergence of proteomics as a distinct discipline is rooted in the recognition that biological function is more directly reflected by proteins—the ultimate products of gene expression—rather than by genes themselves. Consequently, studying the proteome provides deeper insight into cellular function and dynamic biological activity [65]. Advances in mass spectrometry have enabled precise identification and quantification of proteins [66].

7.1 Proteomics Approaches

Proteomics includes various subfields such as structural proteomics, clinical proteomics, and interactomics, each contributing to understanding protein function and interactions (Table 3).

Sub-classes	Function
Structural proteomics	Generally, helps to determine the 3-D structure of the protein and helps to understand the protein-protein interactions and functions.
Clinical proteomics	Aims to diagnose diseases and identify biomarkers of a particular disease
Meta proteomics	To understand the functions and interactions by analyzing the entire set of proteins in a complex microbial community
Interact-omics	Helps to explore the protein-protein interactions and networks also reveals how proteins collaborate
Post-translational modification (PTM) proteomics	Helps to investigate the modifications in proteins after translation glycosylation, phosphorylation, acetylation, etc.

Table 3: Classes of Proteomics [67-71]

Different proteomic approaches help in studying protein structure, function, and disease associations.

Mass spectrometry has become a cornerstone technology in proteomics, enabling both qualitative and quantitative characterization of complex protein mixtures with high sensitivity and specificity. Contemporary research increasingly focuses on determining relative protein expression profiles, which provide deeper insight into biological variability, disease mechanisms, and therapeutic responses. By allowing precise comparison of protein abundances across different conditions, mass spectrometry contributes significantly to the simplification and interpretation of complex protein dynamics. Moreover, it is extensively utilized in the discovery and validation of novel biomarkers, supporting translational research and clinical applications. Structurally, all mass spectrometers comprise three essential components: an ionization source, a mass analyzer, and a detector. The ionization source converts analyte molecules into charged ions, commonly using techniques such as electrospray ionization (ESI) or matrix-assisted laser desorption/ionization (MALDI). These ions are then transmitted into the mass analyzer, where separation occurs based on their mass-to-charge (m/z) ratios. A variety of mass analyzers are available, including quadrupole, ion trap, time-of-flight (TOF), Fourier transform ion cyclotron resonance (FT-ICR), and Orbitrap systems. Advanced hybrid instruments, such as Q-TOF and tandem mass spectrometers (MS/MS), enable multi-stage fragmentation and analysis, thereby enhancing resolution, accuracy, and structural elucidation capabilities.

The operational principle of mass spectrometry relies on the ionization of molecules followed by their transition into the gas phase. The generated ions are accelerated and directed through electric or magnetic fields within the mass analyzer. In many workflows, ions undergo controlled fragmentation within a collision cell, producing characteristic fragment ions that provide structural information. Finally, the detector records these ions, and the resulting spectra are analyzed to determine molecular masses, structural features, and relative abundances.

Overall, mass spectrometry provides a powerful, high-throughput platform for comprehensive proteomic analysis. Its ability to generate detailed molecular information at multiple levels—ranging from intact proteins to peptide fragments—makes it an essential tool for advancing our understanding of biological systems, identifying disease-associated biomarkers, and supporting the development of precision medicine strategies [72–74].

7.2 Quantitative Proteomics

Quantitative proteomics allows comparison of protein expression levels across different conditions and plays a crucial role in biomarker discovery. Quantitative proteomics is important for analysing the dynamic changes in a protein, the identification of biomarkers, and unravelling the molecular mechanisms of a biological process or disease. It includes various techniques, some of which are discussed in Figure 3.

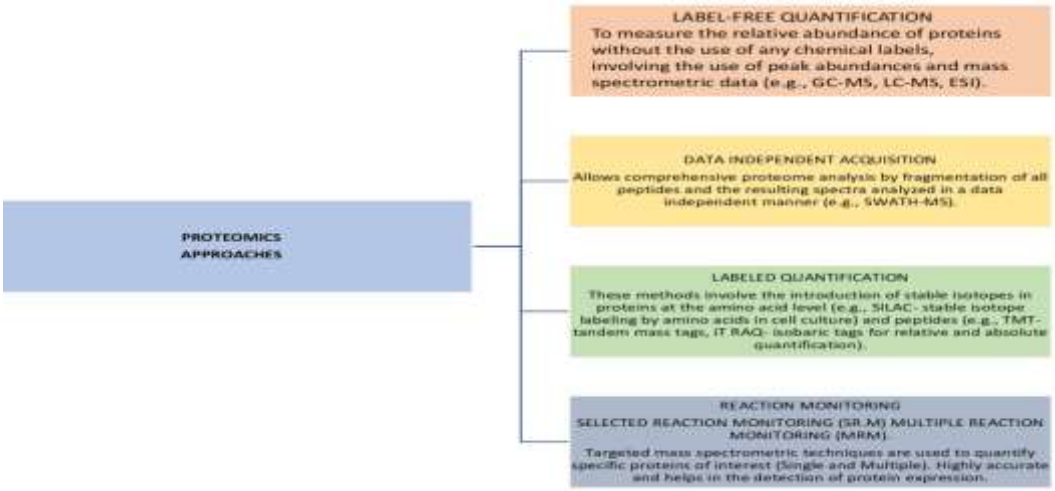

Figure 3: Overview of quantitative proteomics techniques including label-free and labeled approaches.

7.3 Proteomic Biomarkers

Human serum contains a diverse array of proteins that hold significant potential for the early detection of reduced bone mineral density (BMD). Alterations in protein expression are closely associated with the pathophysiology of postmenopausal osteoporosis, highlighting their value as both diagnostic biomarkers and potential therapeutic targets. Several circulating proteins have been implicated in the early stages of BMD loss, supporting their utility in risk assessment and disease monitoring.

For instance, a study by Down et al. identified 20 serum proteins, of which five demonstrated a strong association with hip fracture risk. Notable among these were CD14 (soluble monocyte differentiation antigen), which is involved in lipopolysaccharide-mediated bone resorption, complement factor C7, and sex hormone-binding globulin, all of which are functionally linked to bone metabolism and systemic inflammation [75]. In another investigation conducted in a Chinese cohort, serum exosomal proteomics using liquid chromatography–mass spectrometry (LC–MS) revealed substantial differences in protein expression across groups. A total of 188 proteins were identified in healthy individuals, 224 in osteopenic patients, and 185 in osteoporotic individuals. Among these, 17 proteins were significantly dysregulated in individuals with low BMD, including integrin α2β1, integrin β3, talin-1, and gelsolin, suggesting their involvement in cytoskeletal organization and cell–matrix interactions [76]. Further evidence comes from a study involving 54 postmenopausal women, where tandem mass tag (TMT)-based proteomic analysis identified 1092 proteins. Subsequent validation using targeted approaches confirmed that lysozyme C expression was negatively correlated with BMD, whereas glucosidase and protein disulfide isomerase A5 exhibited a positive correlation. These findings reinforce the complexity of protein regulation in bone metabolism [77]. A more recent cohort study (2022) employing LC–MS-based proteomics examined 20 candidate proteins and reported differential expression patterns between osteopenic and control groups, with eight proteins upregulated and eleven downregulated. Notably, myosin heavy chain 14 showed a progressive increase in expression from healthy to osteopenic to osteoporotic states, indicating its potential as a marker of disease progression. Broadly, proteins related to immunological pathways, coagulation cascades, cytoskeletal structure, and complement systems were among the most significantly altered [78]. Additionally, a study conducted in a Mexican population utilized two-dimensional gel electrophoresis followed by MALDI-TOF/TOF validation in a large cohort of postmenopausal women. Among 27 differentially expressed protein spots, three proteins—ceruloplasmin, gelsolin, and vitamin D-binding protein (VDBP)—were identified as particularly relevant. Importantly, decreased levels of VDBP were strongly associated with low BMD, underscoring its promise as a clinically relevant biomarker [79].

Collectively, these findings emphasize the growing importance of serum proteomics in elucidating the molecular basis of osteoporosis and in identifying reliable biomarkers for early diagnosis, disease stratification, and therapeutic intervention.

8. Future Perspectives and Research Gaps

8.1 Future Perspectives

Future research in osteoporosis is expected to focus on integrating multi-omics approaches to achieve a comprehensive understanding of disease mechanisms. Combining proteomics with genomics and metabolomics may enable identification of robust biomarker panels. Advances in mass spectrometry and bioinformatics will further enhance detection sensitivity and data analysis.

Artificial intelligence and machine learning are likely to play a significant role in analyzing complex datasets and identifying predictive biomarkers. Additionally, personalized medicine approaches based on molecular profiling may improve treatment outcomes.

8.2 Research Gaps

Despite progress, several challenges remain. Lack of standardization in proteomic methodologies limits reproducibility. Many biomarkers require validation in large-scale clinical studies. Longitudinal studies are needed to establish causal relationships. Furthermore, integration of molecular data with clinical practice remains limited. High cost and technical complexity also restrict widespread implementation.

9. CONCLUSION

Osteoporosis remains a major global health concern with significant clinical and economic impact. While current diagnostic and therapeutic strategies have improved patient management, challenges persist in early detection and personalized treatment. Proteomics and omics technologies offer promising avenues for advancing our understanding and improving clinical outcomes. Continued research and technological advancements are essential for translating these findings into routine clinical practice.

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