Review Article

JHSMR Journal of Health Science and Medical Research

Metabolomics in Chronic Kidney Disease: The Emerging Role in Detection and Diagnosis of Mineral and Bone Disorders

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Received 14 August 2024 ● Revised 8 October 2024 ● Accepted 9 November 2024 ● Published online 9 April 2025

Abstract:

Chronic kidney disease (CKD) affects over 10% of the global population and is increasingly prevalent, placing a substantial strain on healthcare systems. In Malaysia, CKD prevalence rose from 9.05% in 2011 to 15.48% in 2018, largely driven by diabetes mellitus. CKD often progresses to mineral and bone disorders (CKD-MBD), a condition starting as early as stage 3a CKD, characterized by disturbances in mineral and bone metabolism that contribute to significant morbidity and mortality. Current diagnostic methods for CKD-MBD, including bone biopsy and imaging, have limitations such as invasiveness, cost, and insufficient sensitivity. Biochemical markers offer some insight but are often not robust enough for the accurate detection and diagnosis of the disease. New protein biomarkers such as sKhloto and FGF23 are restricted by methodological issues and conflicting research outcomes. Metabolomics, the study of small molecule metabolites, provides a promising alternative. By analyzing metabolic profiles in biological samples, metabolomics reveals detailed biochemical changes linked to CKD-MBD. Recent studies have identified certain key metabolites associated with CKD-MBD. Despite some inconsistencies existing across studies, metabolomics, especially when combined with

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J Health Sci Med Res
doi: 10.31584/jhsmr.20251186
www.jhsmr.org

advanced techniques and machine learning, may hold great potential for discovering novel biomarkers for CKD-MBD. This review provides a comprehensive overview of the emerging role of metabolomic profiling in the detection and diagnosis of CKD-MBD. It aimed to enhance the understanding of the disease at a molecular level and explore new avenues for improving the diagnosis and management of this complex condition.

Keywords: biomarkers, chronic kidney disease, CKD-MBD, metabolomics, mineral and bone disease

Introduction

Chronic kidney disease (CKD) is a significant global health problem affecting over 10% of the world's population[1]. Prevalence of CKD is rising each year across many countries. In Malaysia, the prevalence increased over 7 years, from 9.05% in 2011 to 15.48% in 2018, placing a substantial strain on the national healthcare budget. Diabetes mellitus has been identified as a major causative factor that significantly contributes to the prevalence of CKD in Malaysia and worldwide[2].

Most CKD patients are at an increasing risk of progressing to chronic kidney disease-mineral bone disorder (CKD-MBD)[3]. Varying prevalence rates for CKD-MBD have been reported in different studies. Recent data from the Kidney Disease Outcomes Quality Initiative (KDOQI) indicate that the prevalence rate of CKD-MBD is 55%. Meanwhile, the Kidney Disease: Improving Global Outcomes (KDIGO) guideline suggests it may be as high as 86%[4]. CKD-MBD is defined as a systemic disorder of mineral and bone metabolism due to CKD. It usually starts to develop as early as in stage 3a CKD, or when the estimated glomerular filtration rate (eGFR) <60 mL/min/1.73m^2[,5]. The risk of CKD-MBD increases with decreasing kidney function. CKD-MBD manifests in either one or a combination of the following: (i) abnormalities of mineral metabolism (such as calcium, phosphorus, parathyroid hormones (PTH), and vitamin D) (ii) abnormalities in bone metabolism (including bone turnover, mineralization, volume, linear growth, or bone strength) (iii) extra-skeletal calcification (such as cardiovascular or other soft tissue calcification)[6,7]. All these conditions are closely interrelated and conjointly make a major contribution to the high morbidity and mortality of patients with CKD-MBD[7].

The current modalities for the detection of CKD-MBD, such as bone biopsy, radio-imaging and blood biomarkers, face many significant limitations due to their invasiveness, high cost and non-specificity. Considering the debilitating effect of CKD-MBD on the patient's health and the financial burden on healthcare, there is an urgent need for sensitive and accurate biomarkers. Recently, metabolomics has gained relevance in the identification and development of new biomarkers for various diseases, including CKD. Therefore, this review intended to highlight the methods currently employed for the detection and diagnosis of CKD-MBD, discuss their limitations, and present how metabolomic profiling could provide new insight into the early detection of CKD-MBD.

Pathophysiology of CKD-MBD

The progression of CKD-MBD is linked to secondary hyperparathyroidism (SHPT) developed in response to phosphate retention. The retained phosphate leads to hyperphosphatemia and the downregulation of vitamin D (Vit D), resulting in hypocalcemia. Low extracellular calcium ions level is perceived by the calcium-sensing receptors (CaSRs) located on the parathyroid glands, which further up-regulate the production and secretion of PTH. Subsequently, PTH increases the mobilisation of bone calcium and enhances

phosphate excretion. It also inhibits the excretion of calcium and phosphate reabsorption and enhances the synthesis of active Vit D in the kidney[5].

As the function of the kidney progressively worsens, Vit D receptors on parathyroid glands become hypo-responsive to low calcium ions, thus further enhancing the production of PTH. This leads to parathyroid gland hyperplasia, and in some patients, the parathyroid gland undergoes hypertrophy and becomes autonomous[3]. The mechanisms that mitigate these processes remain unclear, and this uncertainty continues to prompt discussion, highlighting the need for further investigation.

A deep understanding of CKD-MBD pathophysiology, particularly at the molecular level is important for improving the detection and diagnosis of the disease. Recently, fibroblast growth factor 23 (FGF23) has been identified as a key player that enhances the understanding of the molecular mechanism of SHPT in CKD. FGF23 is a protein secreted in osteoblasts and osteocytes, requiring αKlotho to bind to its target receptor[5]. In patients with CKD, the accumulation of this protein can lead to auto or paracrine suppression of tissue non-specific alkaline phosphatase (ALP) and the subsequent accumulation of the mineralization inhibitor pyrophosphate, resulting in the impairment of bone mineralization[8]. FGF23 is recognised as a contributor to SHPT in CKD by promoting phosphate excretion and reducing calcitriol production, leading to hypocalcemia and subsequent increased PTH secretion. Additionally, FGF23 binds fibroblast growth factor receptors on the parathyroid gland, stimulating the proliferation of parathyroid cells and further enhancing PTH secretion. Excessive PTH causes the release of calcium from bones, contributing to bone disorders[5,9]. Though the biological effect of FGF23 has been extensively studied, including its independent role in the development of vascular calcification and cardiovascular risk, the regulation of FGF23 synthesis remains unclear as it is yet to be fully elucidated[10]. Figure 1 summarises the pathogenesis, manifestations, and consequences of CKD-MBD.

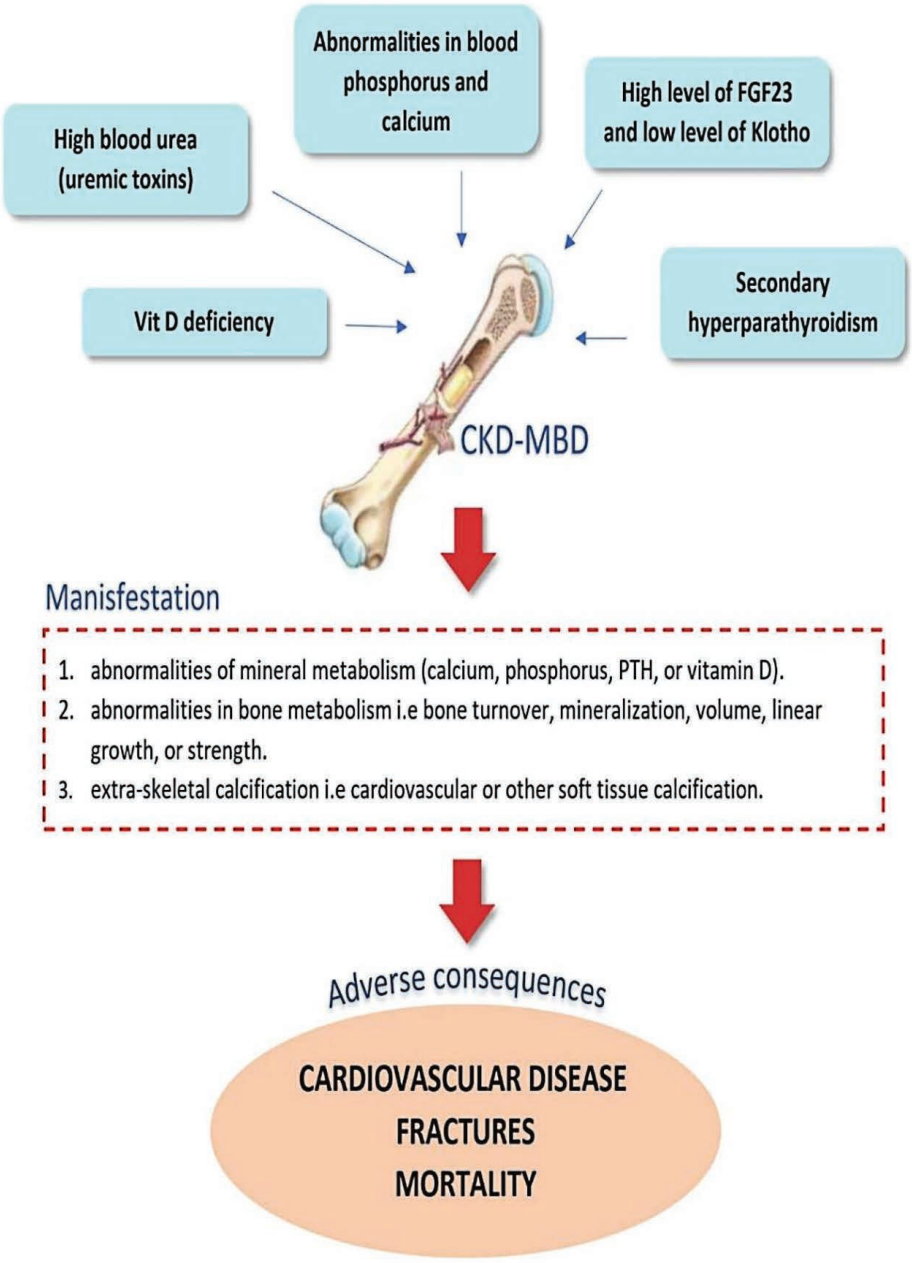
Modalities for the detection and diagnosis of CKD-MBD

Current available approach

Accurate detection and monitoring of CKD-MBD progression in patients with CKD has been a significant challenge and continues to be a critical focus in clinical practice. Figure 2 illustrates the common modalities that are currently used for the detection of CKD-MBD.

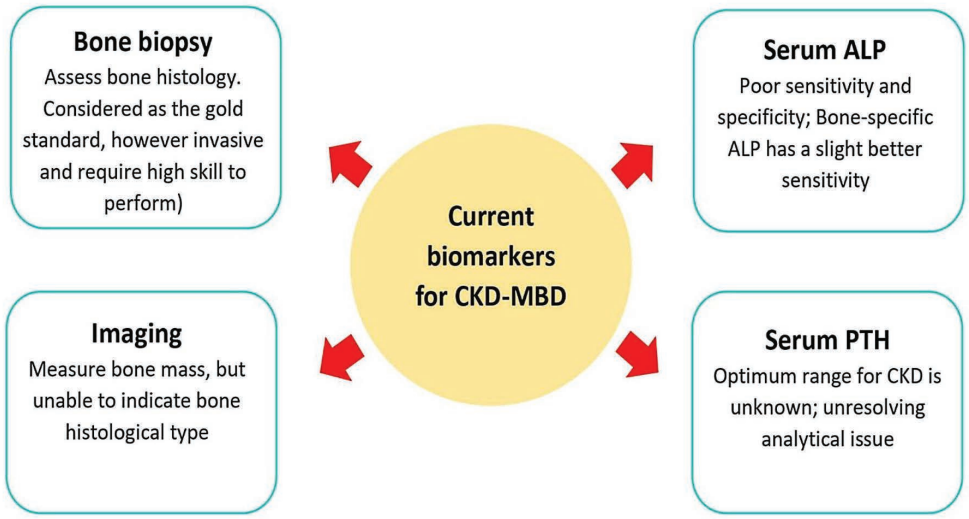
Bone biopsy

Currently, histomorphometry analysis of bone biopsy taken from the iliac crest remains the gold standard for diagnosing bone abnormalities in CKD-MBD[11]. The most common histological type found in CKD-MBD patients is mixed uremic osteodystrophy (MUO), followed by hyperthyroid bone disease and adynamic bone disease[12]. MUO is marked by high bone turnover and mineralization defects, exhibiting characteristics that fall between adynamic and hyperparathyroid bone conditions[13]. Although considered a gold standard, a bone biopsy is rarely performed on patients and is typically performed only at a limited number of specialised centres. A recent European survey of nephrologists specialising in CKD-MBD revealed that only 50% had performed bone biopsies in the past 5 years, with each conducting fewer than 10 biopsies on average. The challenges associated with bone biopsies include labour-intensive and time-consuming procedures, painful sampling, costly histopathological analysis, limited expertise in histopathology, and lack of reimbursement in some countries[14]. Due to the scarcity of data and difficulty in implementation, the 2017 KDIGO guidelines do not recommend the routine use of bone biopsy for the



FGF23=fibroblast growth factor-23, PTH=parathyroid hormone

Figure 1 Summarises the pathogenesis, manifestations, and consequences of chronic kidney disease-mineral bone disorder (CKD-MBD)



CKD–MBD=chronic kidney disease mineral bone disorders, ALP=alkaline phosphatase, PTH=parathyroid hormone

Figure 1 Summarises the pathogenesis, manifestations, and consequences of CKD–MBD

detection of CKD–MBD, except for patients with an uncertain etiology of clinical symptoms and biochemical abnormalities, especially if the results could influence treatment decisions[15].

Imaging technique

Radiography is widely accessible, cost-effective, and the most commonly used imaging technique for diagnosing bone disease. In SHPT, plain X-rays can reveal high bone remodelling and mineralization abnormalities, characterised by the increased bone resorption seen in various bone areas, such as trabecular, endosteal, and cortical, particularly around joints and tendons. Some classical findings that can be observed are the "salt-and-pepper" pattern on skull X-rays and the well-defined lucent lesions or "brown tumours" on X-rays of the pelvis, long bones, or ribs. Despite the widespread availability of this modality, these classical features of SHPT are now rarely encountered in clinical practice[16].

Other useful imaging techniques used in CKD–MBD detection are lateral abdominal plain radiography and echocardiogram. These techniques are considered by KDIGO as reasonable alternatives to computed tomography-based imaging for detecting the presence or absence of valvular calcification[15,16].

BMD testing using dual-energy X-ray absorptiometry (DXA) is another imaging technique that can be used to assess both cortical and trabecular bones. However, calcifications in blood vessels or other tissues can interfere with accurate BMD measurements, leading to an overestimation of bone density at the lumbar spine[16]. Furthermore, this method is unable to indicate histological type or bone turnover, which are also affected in renal bone disease. Consequently, this method is considered reasonable only in situations where a low BMD would prompt additional interventions to reduce falls or when osteoporosis medication is used[7].

Blood biochemical markers

To date, no single biochemical marker singly or in combination is adequately robust to diagnose abnormal

bone turnover in patients with CKD[11]. The most common biochemical markers highlighted in many clinical guidelines are serum PTH and alkaline phosphatase (ALP). The recommendation to use biochemical markers is based on the observation that abnormalities in mineral metabolism often represent early signs of CKD-MBD and can be detected as early as stage 3a of CKD[7]. The KDIGO guidelines recommended using serum PTH in conjunction with total ALP or bone-specific ALP (BALP). The high or low levels of these markers have been shown to correlate with underlying bone turnover and are therefore considered helpful in predicting CKD-MBD[15]. However, the diagnostic utility of these biochemical markers is limited by many factors. Total ALP, though widely available, is not sensitive nor specific in detecting abnormal bone turnover. Conversely, BALP assay is not widely available but is considered more specific and sensitive compared to total ALP. However, the clinical value of cut-off levels and treatment targets for BALP in CKD-MBD management, including cardiovascular and fracture prevention, is unavailable and yet to be established through studies[17].

The use of PTH is complicated by the unresolved issues of different PTH assays (intact vs bio-intact, second vs third generation) that contribute to variable performances and reference ranges. This imposes significant difficulties and incomparability in interpreting the results. To overcome the limitations of PTH assay, clinicians are recommended to observe the rising or declining trends of serial PTH measurements for detecting abnormal bone turnover rather than looking at one single value[7]. However, the time taken for sufficient observations to take place may hinder early detection, causing a considerable delay in the treatment[15]. In addition, studies comparing serum PTH with bone histomorphometry, bone CT, and DXA revealed that PTH alone did not present a good performance for diagnosing bone turnover; thus, further impeding its role in the detection and diagnosis of CKD-MBD[18].

Bone turnover markers

In addition to biochemical markers, bone turnover markers (BTMs) such as procollagen type 1 amino-terminal propeptide (P1NP) and carboxy-terminal cross-linked telopeptides of type 1 collagen (CTX) have been proposed for diagnosing bone disease in CKD[19]. P1NP is released when type 1 collagen is deposited in the bone matrix, making it a reliable indicator of bone formation[20]. In contrast, CTX is produced when type 1 collagen is broken down by cathepsin K during the process of bone resorption, which makes it a marker for bone resorption[18].

The clinical utility of P1NP and CTX is currently limited. Research indicates that collagen-based markers do not provide significant advantages over BALP and PTH for assessing bone turnover in patients with advanced CKD[18]. Although they demonstrate comparable diagnostic accuracy, these bone markers are less widely available and more influenced by various biological factors, including fractures, immobilization, circadian rhythms, exercise, diet, and high intra-individual variability[21]. Additionally, their effectiveness in predicting bone mineral density and bone histology has been inconsistent between studies. Consequently, the KDIGO guidelines do not recommend using these markers for diagnostic purposes[15]. As a result, many laboratories do not offer these tests, and most nephrologists do not incorporate them in their clinical practice, at least at present[22].

Protein biomarkers

Considering the role of FGF23 in the pathogenesis of bone disorder in CKD, this protein has been proposed as a useful biomarker for CKD-MBD detection. In individuals with CKD, FGF23 levels often rise before serum calcium, phosphorus, or PTH levels change[23]. Various experimental models of CKD show an increase in FGF23 preceding elevation of PTH. For instance, a study involving adults with normal kidney function revealed that PTH levels started to rise when eGFR fell below 126 mL/min/1.73 m^2, whereas

FGF23 levels began to increase when eGFR dropped below 102 mL/min/1.73 m^2[24]. Hence, elevated levels of FGF23 can be considered among the earliest detectable biomarkers for CKD-MBD[23]. However, routine analytical measurement of FGF23 for clinical practice remains a challenge and has only been partially addressed. While variability in the results due to the sample processing and collection can be minimised through standardized pre-analytical procedures, the lack of standardization among available assays continues to pose a significant analytical challenge, leading to inconsistencies and the non-comparability of results across different methods and laboratories[25]. Furthermore, the data regarding the bioactivity of FGF23 has remained insufficient, further hindering the development of international standards and accurate measurement methods[26]. Additionally, determination of clinically significant levels of FGF23 in CKD patients is also challenging due to the high intra- and inter-variability of the FGF23[27]. All these factors collectively limit the routine measurement and use of FGF23 as a biomarker for CKD-MBD in clinical practice.

Following the discovery of αKlotho as a co-receptor for the FGF23 receptor in the kidneys, significant interest has developed regarding its role in CKD-MBD. Research indicates that serum and urinary αKlotho levels, specifically its soluble form (sKlotho), decrease as CKD progresses. Several contributing factors for such decreases have been identified including high albuminuria, hyperphosphatemia, and epigenetic changes in the Klotho gene promoter[28]. Conversely, subsequent studies showed no correlation between sKhloto and renal function and adverse outcomes in patients with CKD[29]. These contradictory findings have created considerable uncertainty regarding the reliability of sKlotho as a biomarker for CKD-MBD. The inconsistency in the study findings likely stems from the diverse methodologies used across the studies, including variations in CKD experimental models and the inclusion of a limited number of patients with poorly characterised kidney or vascular damage.

Metabolomic analysis

Metabolomics technology has recently gained popularity as a method for identifying potential disease biomarkers and understanding the disease mechanism. Metabolomics is an important 'omic' approach to analyse multiple small molecule metabolites less than 1 kDa in various biological specimens, including urine, blood, saliva, tissues, and breath exhalate[30,31]. These metabolites are considered as markers of various biological processes that connect cellular traits to their genetic makeup and provide insight into how specific genes are regulated and how environmental factors can change body metabolism. Through the analysis of metabolic profiles in both normal and pathological conditions, metabolomics allows for a thorough characterisation of diseases at the metabolic level[30].This makes metabolomic analysis a promising technique for identifying biomarkers and uncovering the metabolic pathways related to various diseases[31].

Generally, metabolomic profiling can be studied through either an untargeted analysis of a broad range of unknown metabolites or a targeted quantification of specific, pre-defined metabolites. Untargeted metabolomics explores a wide range of unknown metabolites without prior bias. This discovery-based approach enables the global detection of all metabolites linked to phenotype information, allowing for the identification of novel metabolites and pathways. In contrast, targeted metabolomics involves analysing a predefined set of metabolites and tends to be more sensitive and reproducible relative to untargeted approaches. Nevertheless, both approaches seek to identify any distinguishing features that can differentiate between the sample groups; thus, aiding in the development of new diagnostic or staging biomarkers and exploring novel hypotheses regarding a disease's etiopathogenesis[32].

More recently, untargeted metabolomic methods have been coupled with machine learning in order to enhance the identification of signature metabolites using biostatistics[32,33]. In a study of peadiatric CKD patients, this approach successfully identified metabolite signatures related to the underlying causes of the disease. The new finding offers valuable insights into biochemical processes and helps generate hypotheses for future research[33]. Although this field is relatively new, it may hold great potential for application in the study of CKD-MBD.

There are several types of analytical tools available to perform metabolomic analysis, with the most common being nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS). Metabolomics mass spectrometry-based has the advantage of quickly identifying small molecules, and thus enhancing the understanding of metabolic mechanisms in diseases. Its versatility and sensitivity are enhanced when paired with separation techniques like liquid chromatography (LC-MS), gas chromatography (GC-MS), and capillary electrophoresis (CE-MS)[34]. LC-MS is particularly valuable because it can accurately separate and identify metabolites in a shorter time, making it the best option for analysing many clinical samples simultaneously with high sensitivity[30].

Metabolomics of CKD-MBD

Lack of accurate detection approaches and the unclear molecular basis of CKD-MBD pathophysiology may prevent patients from receiving precise diagnoses and timely personalized treatment. Therefore, searching for a more accurate biomarker for detecting the disease and understanding the disease pathophysiologic mechanism through appropriate methodology is critical.

Metabolomics profiling is increasingly recognised for its role in studying a broad spectrum of diseases, such as cardiovascular conditions, cancers, diabetes mellitus, and CKD[35–38]. Recently, there has been a growing interest in applying metabolomics to investigate mineral and bone disorders associated with CKD. Several studies have reported promising findings in this area.

Signature metabolites of CKD-MBD

In 2015, Wu et al. conducted a metabolomic study involving CKD subjects with SHPT receiving maintenance peritoneal dialysis. In the study, serum iPTH cut-off level >300 pg/mL was used to define SHPT. Based on the metabolomic analysis using ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF/MS), 32 unique metabolites were identified in those with SHPT, with 30 of them found elevated and 2 (cytidine and L-phenylalanine) downregulated[39] (Table 1).

Key metabolites highlighted in this study include L-phenylalanine, N-(1-Deoxy-1-fructosyl) tryptophan, dopamine glucuronide, N-acetylserotonin, and ploryl-tyrosine. These metabolites serve as either substrates or products in various metabolic processes. Other metabolites highlighted in this study were those from the amino acid pathway such as glycylprolylhydroxyproline and glutaminyl-hydroxyproline. These hydroxyprolines are known to be released during degradation of bone collagen[40]. However, because they can also be found in other tissues such as skin, they are considered an unspecific index of bone turnover in CKD-MBD[41].

Additionally, TCA cycle intermediates related to energy metabolism, including diethyl fumarate, (R)-2-methylmalate, isopropyl citrate, glutamyl-glutamate, and N-acetylaspartylglutamic, were also identified to be significantly upregulated in SHPT. This upregulation is likely related to the increased energy demands of bone cells, resulting from elevated PTH levels. Nonetheless, the specificity of TCA cycle intermediate as CKD-MBD biomarkers is limited since these metabolites can be elevated in various diseases[39,42], such as neurometabolic

Table 1 Signature metabolites in CKD patients associated with SHPT in different studies

Study	Metabolomic method	Subjects	Differential metabolites
Wu et al., 2015	UPLC–Q–TOF/ MS	19 CKD patients with iPTH >300 pg/ml (SHPT group) and 19 CKD patients with iPTH between 150–300 pg/ml (control group)	*Elevated:* N–(1–Deoxy–1–fructosyl)–tryptophan, N–acetylserotonin glucuronide, Dopamine glucuronide, Prolyl–tyrosine, Glycylprolylhydroxyproline, Aminohippuric acid, 2–phenylglycine, 4–carboxyphenylglycine, N–(3–Indolylacetyl)– L–isoleucine, Glutaminyl–hydroxyproline, Diethyl fumarate, Isopropyl citrate, (R)–2–methylmalate, Glutamyl–glutamate, N–acetylaspartylglutamic acid, (R)–pantothenic acid 4–O–b–D–glucoside, Ethyl sorbate, Folic acid, Indolpyruvate, Methyl sorbate, N–acetylgalactosamine 4,6–disulfate, Cortisol 21–sulfate, Uridine, Androsterone glucuronide, LysoPC (14:0), 2–O–methyl–L–fructose, 3–Ethylpyridine, Ascorbate 2 –sulfate, Adrenosterone, N–acetylgalactosamine 4–sulfate *Decreased:* Cytidine and L–phenylalanine
Shen et al., 2019	UPLC–MS	15 CKD patientts on maintenance dialysis whose iPTH level >600 pg/ml (SHPT group) and 15 CKD patients on maintenance dialysis who had achieved iPTH <150 pg/ml following parathyroidectomy (control group)	*Elevated:* Allyl isothiocyanate, D–aspartic acid, L–phenylalanine, Inosine, Biotin, 3–Nitrotyrosine, Pyrimethamine, Menadione *Decreased:* D–galactose and Indoleacetaldehyde.
Gan et al., 2024	Untargeted UPLC–MS/MS	3 groups i.e. 85 CKD subjects with normal iPTH, 76 CKD subjects with SHPT and 67 healthy controls. SHPT were defined according to the CKD stages, with iPTH level set as follows: >70 pg/mL in stage 3 CKD, >110 pg/mL in stage 4 CKD, and >300 pg/mL in stage 5	*Elevated:* N–Acetyl D–tryptophan, 5–Hydroxytryptophan, Tryptophyl–Leucine, 2 Picolinic acid, Nicotinic acid, Lysyl–Phenylalanine, Phenylacetyl–L–glutamine, 3–Indole carboxylic acid glucironide, Indoxyl sulfate, Indoxyl glucuronide, Kynurenic acid, Hippuric acid, Creatinine, Uridine, Biotin, L–acetylcarnitine, O–Butanoylcarnitine, O–Decanoyl–L–carnitine, L–Palmitoylcarnitine, Stearoylcarnitine, Linoleyl carnitine, Oleoylcarnitine, LysolPC(O–18:0), SM d35:3, PA (18:1(9Z)/22:4(72,10Z,13Z,16Z)), PE(13:0/16:1(9Z)), PE(14:1(9Z)/P–16:0), PS(P–16:0/12:0), LDGTS 7:0 *Decreased:* L–Tryptophan and (R)–(+)–1–phenylethylamine

UPLC–Q–TOF/MS=ultra performance liquid chromatography quadrupole time–of–flight mass spectrometry, iPTH=intact parathryoid hormone, CKD=chronic kidney disease, LysolPC=Lysophosphatidylcholine, SHPT=secondary hyperparathyroidism

disorders and tumours[42]. This study also identified androsterone, nucleosides (e.g., uridine, cytidine), lipids (e.g., ethyl sorbate and methyl sorbate), vitamins (e.g., ascorbate and pantothenic acid) and folate as metabolites that were significantly altered in high–PTH patients[39]. However, the mechanisms behind the altered levels of these metabolites, especially androsterone and vitamins, are not well–documented in the literature, emphasizing the need for further research. Based on the above findings, the authors suggest that, in addition to the well–established mineral and hormonal changes such as hyperphosphatemia, hypercalcemia, hyperparathyroidism, and active vitamin D deficiency, there are unidentified metabolic pathways related to protein, amino acid, energy, and steroid hormone

metabolism that may also play a role in the onset of CKD-MBD[39].

In 2019, Shen et al. subsequently conducted a metabolomic study in SHPT, involving 15 CKD subjects on maintenance dialysis whose iPTH level was >600 pg/ml (SHPT group) and 15 CKD subjects on maintenance dialysis who had achieved iPTH <150 pg/ml following parathyroidectomy (control group). Metabolomic analysis utilising ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) successfully identified 10 metabolites that accurately differentiated CKD subjects with SHPT from the normal control group (Table 1). At a cutoff of 0.2708, the combined metabolites demonstrated good sensitivity and specificity of 0.93 and 0.8, respectively, with an area under the curve of 0.947[43].

Notably, there were 5 metabolites (i.e. N-acetylaspartylglutamic acid, indolepyruvate, uridine, adrenosterone, and L-phenylalanine) considered common when compared to the earlier study,[43] emphasizing the alteration of protein, energy and steroid metabolism in the pathogenesis of CKD-MBD. The trend of these metabolites was consistent between these studies, except for L-phenylalanine, which was found to be upregulated and contradicted the findings of the earlier study.

The most recent metabolomic study of SHPT was conducted in 2024, involving pre-dialysis CKD subjects of stages 3–5[44]. This study involved a larger sample size and employed a more versatile method for metabolomic analysis, i.e., untargeted ultra-performance liquid chromatography-tandem mass (UPLC-MS/MS). The metabolites were compared across 3 groups (i.e. 85 CKD subjects with normal iPTH, 76 CKD subjects with SHPT, and 67 healthy controls). SHPT was defined according to the CKD stages, with the iPTH level set as follows: >70 pg/mL in stage 3 CKD, >110 pg/mL in stage 4 CKD, and >300 pg/mL in stage 5. The analysis showed that the differential metabolites were primarily enriched in 4 key pathways from the Kyoto Encyclopedia of Genes and Genomes (KEGG): tryptophan phenylalanine and tyrosine biosynthesis; sphingolipid metabolism; glycerophospholipid metabolism; and phenylalanine metabolism. Among the 31 identified metabolites, 2 (tryptophan and phenylethylamine) were significantly downregulated, while the remaining 29 metabolites, which were mainly derived from amino acids and their derivatives, lipids, carnitine, and uremic toxins, were significantly upregulated (Table 1). All the lipid metabolites were positively correlated with the levels of urea, creatinine, cystatin C and triglycerides, and negatively correlated with eGFR and cholesterol levels, indicating the presence of an association between the differential lipid metabolites with reduced renal function and abnormal lipid levels in patients with SHPT[44].

The findings from existing metabolomic studies indicate that the biosynthesis of phenylalanine and tryptophan is the most frequently altered in SHPT. Tryptophan is an essential amino acid that is metabolized via the kynurenine, serotonin, and indoles pathways[45]. Studies reported that the degradation products of tryptophan, such as kynurenic acid and 5-hydroxytryptophan, have detrimental effects on bone health, reduce bone mineral density, and increase the risk of fractures[44,46,47]. Elevated kynurenic acid levels also suppress cellular metabolism in osteoblasts and decrease their numbers[48]. Additionally, indoxyl sulfate, another metabolite of tryptophan, exhibits proinflammatory properties. Its effects include reducing bone mineral density and bone formation, stimulating osteoblast apoptosis, suppressing osteoclast differentiation, and inducing skeletal resistance to PTH in patients with CKD[49,50].

L-phenylalanine is also an essential amino acid vital for the synthesis of protein, melanin and tyrosine. Both tryptophan and L-phenylalanine have been shown to play an important role in calcium homeostasis and phosphate regulation[51]. These amino acids act as an allosteric activator of the CaSRs, enhancing the sensitivity of parathyroid cells

to calcium. This leads to increased calcium mobilization and decreased secretion of PTH[52]. Thus, significant upregulation of L-phenylalanine in patients with SHPT may function as a negative feedback mechanism aimed at lowering elevated PTH levels[43].

Another significant metabolite found in these studies was indole pyruvate, an endogenous metabolite produced from the metabolism of dietary tryptophan by the gut microbiota[45]. Compared with other tryptophan metabolites, such as indoxyl sulfate, the role of indole pyruvate in the pathogenesis of SHPT or CKDMBD is less well studied, highlighting the need for further research in this area.

Other metabolites, such as uridine and biotin, also have been consistently reported across the studies. Uridine is a pyrimidine nucleoside that plays a key role in various biological processes, including the synthesis of macromolecules, regulation of circadian rhythms, modulation of the inflammatory response, antioxidant activity, and the ageing process[53]. Biotin, in contrast, is a water-soluble vitamin that serves as a cofactor for enzymes involved in carboxylation reactions. As such, it is vital in fatty acid metabolism, amino acid metabolism, carbohydrate metabolism, and various other cellular processes[54]. Despite the significant dysregulation of these metabolites, the mechanisms underlying their alteration are not well documented in the literature, emphasizing the need for further research.

While factors such as geographical region and ethnicity can influence metabolomic profiles[55,56], they may not significantly explain the variations in differential metabolites observed, especially considering that the studies were conducted in the same country (China). The discrepancy of the differential metabolites across these studies could be attributed to other factors, such as the dietary pattern or nutritional therapy of the subjects[57]. For instance, most CKD subjects, especially those in the advanced stage, followed restricted diets in order to slow the disease progression. Similarly, subjects with SHPT who underwent parathyroidectomy may have also adhered to strict dietary controls before the surgery. Therefore, the subjects' current nutritional and metabolic status is an important variable influencing the differential metabolites identified in the studies. Treatments, such as phosphate binders and Vit D analogues, commonly prescribed to advanced CKD subjects and those on dialysis could also alter the metabolic pathway and contribute to variations in differential metabolites[58]. Another factor is the CKD stage of the subjects involved in the studies. A metabolomic study conducted regarding the various CKD stages reported a major difference in metabolite profiles among the subjects; thus revealing the stage-specific biomarkers of CKD[38]. Additionally, lifestyle, endogenous metabolism, gut microbial metabolism and psychological state are other influential factors contributing to variations in the metabolomic profiles[58]. Lastly, variations in sample sizes may have also contributed to the discrepancies in the differential metabolites observed across studies. Using smaller sample sizes can result in findings that lack sufficient power to identify differential metabolites between groups. This is evident from the findings of the existing metabolomic studies, which show that those with larger sample sizes tend to identify a greater and more varied set of significant metabolites, compared to studies with smaller sample sizes[39,43,44]. Since metabolomic research aims to identify a specific set of metabolites that play a significant role in distinguishing samples between 2 different groups, designing the study with a suitable sample size is critical, if reliable and statistically significant metabolites are to be obtained[59].

Based on the existing metabolomic studies in SHPT mentioned above, the subjects with SHPT were identified based on their serum intact PTH levels. While intact PTH is recommended as a standard assessment in CKD-MBD, it does not provide information about bone histological types,[15,60] making it less specific for evaluating

renal osteodystrophy. To address this limitation, a study was conducted to correlate the metabolomic profiles with bone histomorphometry in CKD subjects undergoing hemodialysis in order to identify metabolites related to bone turnover, volume, and mineralization[61]. It involved 51 subjects and utilised nuclear magnetic resonance (NMR) spectroscopy for metabolomic analysis. The study hypothesised that dimethylsulfone, citrate, and glycine were associated with bone turnover, whereas ethanol and carnitine were linked to bone mineralization and bone volume, respectively. However, further validation of these findings with a larger sample size and more versatile methods, such as LC–MS or MS, is needed in order to capture additional metabolites and produce more robust results.Top of FormBottom of Form

Conclusion

The rising prevalence of CKD and its associated mineral and bone disorders underscores the need for advanced diagnostic and monitoring tools. While traditional methods like bone biopsy and imaging techniques have limitations, metabolomics offers a promising alternative by providing detailed insights into metabolic alterations linked to CKD–MBD. Recent studies have identified various signature metabolites and metabolic pathways associated with SHPT; thus, enhancing the understanding of its pathophysiology over CKD–MBD. Despite some inconsistencies in study findings, the application of metabolomics in conjunction with advanced analytical techniques coupled with machine learning holds significant potential for the identification of novel biomarkers in improving the early detection, diagnosis, and personalised management of CKD–MBD. More research with a meticulous and systematic approach is necessary in order to address the challenges posed by the variability in metabolite levels. This encompasses designing research with appropriate study designs and rigorous protocols, including standardised subject selection across diverse populations. Additionally, leveraging advancements in metabolomic technology is equally crucial to developing more effective strategies for detecting and managing this complex disease.

Authors' contributions

Ms. Faten Noorzafarina conducted article searches for this review, while Dr. Tuan Salwani and Dr. Lim Vuanghao reviewed and provided critical feedback for the improvement of the manuscript. Dr. Rohayu reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding sources

This article was funded by the Malaysia Ministry of Higher Education Fundamental Research Grant Scheme (FRGS) grant no. FGRS/1/2022/STG02/USM/03/1.

Conflict of interest

There are no conflicts of interest to declare.

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