Correlation of Serum Parathyroid hormone, Serum Osteocalcin and Serum Tartrate resistant acid Phosphatase (TRACP) 5b with Bone mineral disease in Chronic Kidney Disease patients

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Abstract:

Introduction: CKD is a major cause of fatality in India as described as kidney damage or decreased kidney function for three months or longer, regardless of the cause. The retention of phosphorus decreases the levels of calcitriol within the parathyroid gland that give rise to increased iPTH secretion as well as increased parathyroid growth, skeletal resistance towards iPTH and hypocalcaemia. Also Serum osteocalcin levels provide a non-invasive marker of osteoblast activity and bone formation. Also Tartrate-resistant acid phosphatase (TRACP) 5b is secreted into the circulation from osteoclasts during bone resorption and has been proposed as a bone resorption marker with an increased serum level that is independent of renal dysfunction.

Objectives: We wanted to evaluate the correlation of serum iPTH, Osteocalcin and (TRACP) 5b levels with bone mineral disease in CKD patients as a potential biomarker.

Methods: This study was conducted in 300 subjects out of which 150 were CKD patients (cases) and 150 were healthy subjects (controls). 3 ml peripheral venous blood was collected and supernatant serum as used for the analysis of Serum iPTH, Serum Osteocalcin and Serum TRACP5b by (ELISA) method.

Results: We found significantly increased levels of serum iPTH (OR-3.07, $P < 0.0001^{***}$), serum Osteoclacin (OR-7.12, $P < 0.001^{**}$) and (TRACP) 5b (OR-5.62, $P < 0.0001^{***}$) in CKD patients as compared to controls.

Conclusions: Bone mineral disease in chronic kidney disease patients can be detected at early stage by the use of noninvasive methods of estimation of Serum iPTH, Serum Osteocalcin and (TRACP) 5b levels and can be used as prognostic (biomarker).

Keywords: Parathyroid hormone, Osteocalcin, (TRACP) 5b, Bone mineral disease, Chronic kidney disease.

Introduction:

Regardless of the cause, kidney damage or impaired kidney function lasting three months or longer is referred to as chronic kidney disease [1]. The broad clinical conditions known as mineral bone disorder and chronic renal disease are caused by a systemic malfunction of

mineral and bone metabolisms [2]. Biochemical and hormonal alterations that dramatically increase the risk of bone fractures, cardiovascular events, mortality, and the progression of chronic kidney disease characterize the mineral and bone problems, also referred to as the acronyms for chronic renal disease [3]. The gold standard for assessing and diagnosing renal osteodystrophy is bone biopsy, and in order to identify bone pathology, it is recommended to categorize cases according to bone turnover, mineralization, and volume [4]. Trabecular bone biopsy for histomorphometry analysis is the gold standard for diagnosing and monitoring mineral bone disorders and chronic renal disease [5]. In osteoporosis, a common metabolic disorder of the skeleton, low bone mass and microarchitecture degeneration of bone tissue increase the fragility and fracture susceptibility of bones [6]. The bones may deteriorate to the point where they could break spontaneously or in reaction to minor stress. The person may have ongoing pain and a decreased ability to perform daily activities after the fractured bone heals [7]. High turnover metabolic bone disease and chronic renal disease High-turnover bone disease is caused by SHPT development. It has long been known that parathyroid gland hyperplasia and elevated blood PTH levels occur early in the course of chronic kidney disease (CKD) [8]. Low-turnover metabolic bone disease in chronic renal illness is another name for adynamic bone disease. An unusually slow rate of bone formation is the hallmark of low-turnover bone disease, which is commonly observed in patients with renal disease, especially those undergoing dialysis. Hypocalcemia, skeletal resistance to PTH action, retention of phosphorus, decreases in calcitriol levels, and intrinsic changes in the parathyroid gland that result in increased parathyroid gland development and PTH production [9]. Serum osteocalcin, a 49-amino acid protein that binds calcium and is dependent on vitamin K, is released by mature osteoblasts. It is a specific biomarker of bone turnover and production. Serum osteocalcin plays a crucial role in preventing the mineralization of cartilage and the crystallization of hydroxyapatite [10]. The amount of serum osteocalcin in the blood has been found to be a non-invasive indicator of bone formation because osteoblasts produce it during the formation of new bone [11]. Serum tartrate-resistant acid phosphatase 5b, which osteoclasts release into the bloodstream during bone resorption, has been proposed as a marker for this process [12]. One bone resorption marker whose level is unaffected by renal impairment is serum tartrate resistant acid phosphatase 5b, which is released into the bloodstream by osteoclasts during bone resorption [13]. An osteoclastic enzyme called tartrate-resistant (type 5) acid phosphatise (TRACP) is released during bone resorption [14]. An efficient indicator of the rate of bone resorption in women with osteoporosis, serum TRACP levels are elevated in a number of diseases involving increased osteoclastic activity [15].

Objectives

We wanted to evaluate the correlation of serum iPTH, Osteocalcin and (TRACP) 5b levels with bone mineral disease in CKD patients as a potential biomarker.

Methods:

This study was a case control study conducted in Department of Biochemistry, Pacific Medical College and Hospital, Udaipur, (Rajasthan) India. Study was carried out after obtaining ethical clearance from Institutional Ethics committee, Pacific Medical College and Hospital, Pacific Medical University, Udaipur, (Rajasthan) India. Study involved 300 subjects, 150 healthy subjects and 150 chronic kidney disease patients attending the Dialysis Unit, Nephrology OPD/IPD and Department of Medicine in Pacific Medical College and Hospital, Udaipur, Rajasthan. The study design is shown as follows [Figure 1].

Inclusion Criteria:

Chronic Kidney Disease Patients (stage I- stage V) before starting dialysis therapy.

Subjects between 18–65 years age group were considered.

Exclusion criteria:

Acute infections.

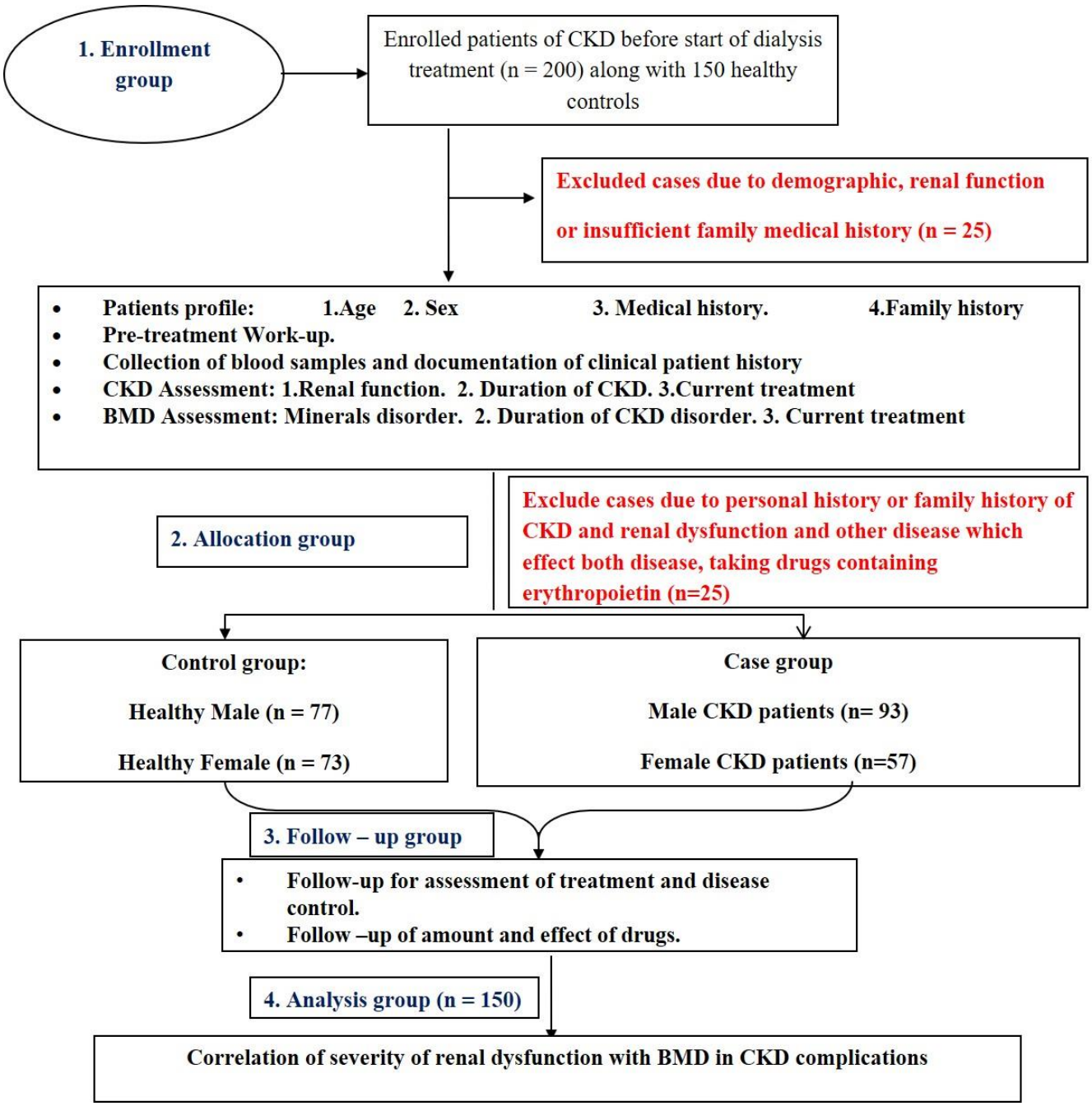
Malignancy.

Chronic liver disease.

Thyroid gland dysfunctions.

Myocardial infarction.

Figure 1: Study design.



Method of Analysis:

3 ml peripheral blood samples were collected from all participants both patients and healthy controls. The blood samples were centrifuged at 4000 rpm for 15 minutes. The 50µl of centrifuge serum was used in a fully auto-analyzer Roche cobas e 801 to measure the serum parathyroid hormone, which was estimated by Chemiluminescence Immunoassay (CLIA). The Serum Osteocalcin and Serum Tartrate resistant acid Phosphatase 5b (TRACP) 5b were estimated by Enzyme-linked immunosorbent assay (ELISA) semi auto-analyzer ELISA Reder Bio-Red PR 4100, Elbscience kit and lot no. E-EL-H1343. The serum was stored at -70°C until assayed.

Statistical Analysis:

All the parameter of case and control were analyzed for mean and standard deviation. The results were expressed as Mean ± standard deviation. The student t-test was used and a p-value < 0.05 was considered statistically significant. Pearson correlation coefficient was used to find the correlation between the level of Serum Parathyroid hormone, Serum Osteocalcin, and Serum Tartrate resistant acid Phosphatase 5b (TRACP) 5b with Bone mineral disease level in chronic kidney disease patients as compared to controls as well as pre and post dialysis of patients. Data was analyzed using Statistical software i.e. Statistical Package for Social Sciences (SPSS) version 21.0. After analysis of data distribution patterns appropriate statistical tests was utilized for analyzing measures of central tendency, dispersion and odds ratio.

Results:

The study included subjects 300 participants among them 150 were Pre dialysis patients with chronic kidney disease with ages ranged from 18 to 65 years with a mean of 48.26±10.49 years and 150 were heathy controls. There were 150 Case groups (male 77% and female 73 %) and 150 Control groups (male 93% and female 53%). Older age dialysis patients with CKD were more common i.e. in the 50–60-year age group. The p-value of 0.05 was considered significant to compare the pattern of renal profile between the two groups [Table 1].

Table 1: Comparison of Bone mineral disease and Blood parameters between healthy controls and CKD cases

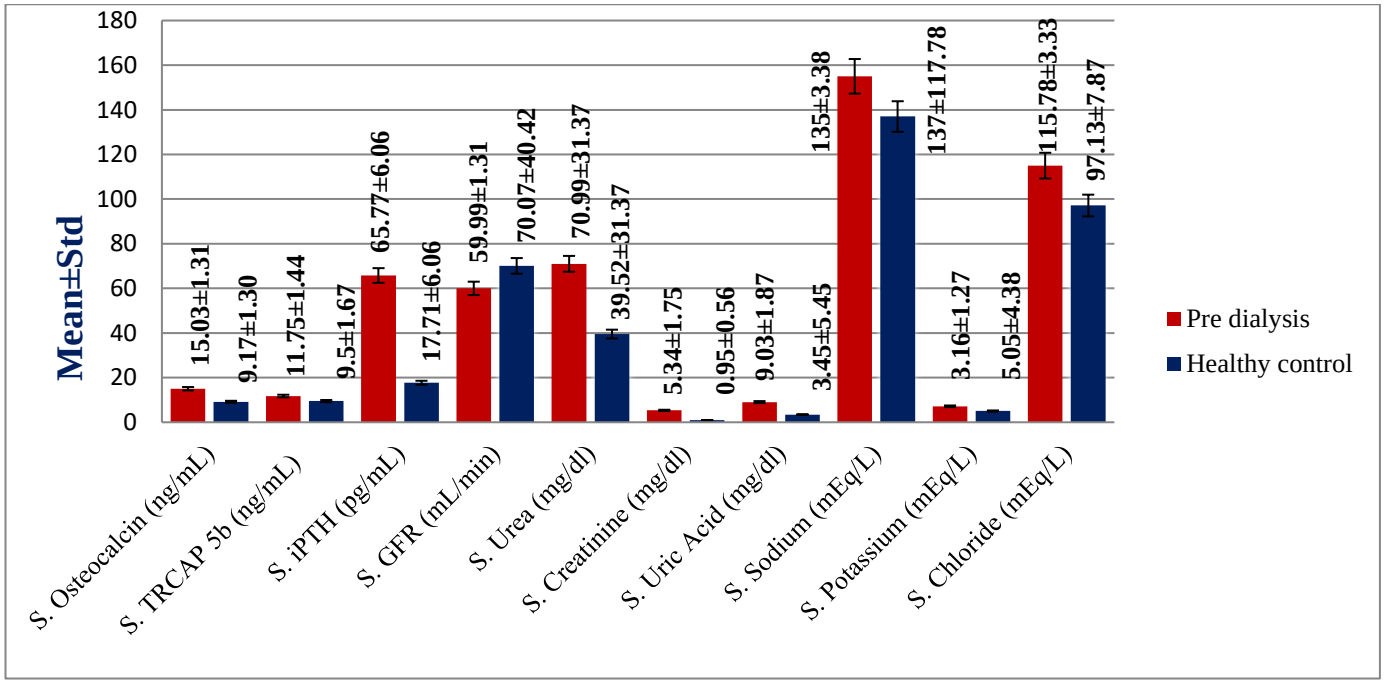
S. No.	Parameters	CKD Cases (Mean ± S.D) (150)	Healthy Controls (Mean ± S.D) (150)	OR	P Value
01.	S. Osteocalcin (ng/ml)	15.03±1.31	9.17±1.30	7.12	0.0012(**)
02.	S. TRACP 5b (ng/ml)	11.75±1.44	9.5±1.67	5.62	0.0001(***)
03.	S. iPTH (pg/ml)	65.77±6.06	17.71±5.20	3.07	0.0001(***)
04.	S. eGFR (ml/min)	70.07±40.42	59.99±1.31	5.65	0.0001(***)
06.	S. Urea (mg/dl)	70.99±6.01	39.52±31.37	3.87	0.0001(***)
07.	S. Creatinine (mg/dl)	5.34±1.75	0.95±0.56	7.85	0.0014(**)
08.	S. Uric Acid (mg/dl)	9.03±1.87	3.45±5.45	5.78	0.0001(***)

09.	S. Sodium (mEq/L)	155±3.38	137±117.78	5.90	0.0571
10.	S. Potassium (mEq/L)	7.16±1.27	5.05±4.38	5.80	0.0005(***)
11.	S. Chloride (mEq/L)	115.71±3.33	97.13±7.87	7.85	0.0354(*)

*P value<0.05, **P value <0.005, ***P value <0.0005 as considered significant

We observed significantly increased levels of S Osteocalcin (15.03±1.31) , TRAPCP 5b (11.75±1.44) and S iPTH (65.77±6.06) in CKD patients as compared to control,. Urea, Creatinine, Uric acid, eGFR, Potassium and Chloride were also significantly elevated in CKD patients as compared to controls [Table 1]. All these observations were was statistically significant (P < 0.05). Serum Sodium was however elevated in CKD patients but was non-significant [Figure 2].

Figure 2: Comparison of Bone mineral disease and Blood parameters between healthy controls and CKD cases



Discussion:

Increased fibroblastic growth factor 23 (FGF23), vitamin and calcitriol deficiency, hyperparathyroidism, and disordered phosphate and calcium metabolism are some of the abnormalities in mineral metabolism that cause CKD-MBD, or changes in bone, a multifactorial disorder [16]. Osteitis fibrosa cystica, low turnover or adynamic bone disease, mixed uremic osteodystrophy and osteomalacia have historically been the four main forms of bone disease that occur in chronic kidney disease (CKD) [17]. Osteitis fibrosa is primarily

caused by hyperparathyroidism, which appears relatively early in chronic kidney disease [18].High turnover with peritrabecular fibrosis, active osteoclasts and an increase in multinucleated osteoclasts, woven bone, crossed tetracycline labels, increased cancellous bone volume but decreased cortical thickness, and intratrabecular tunneling are the classic signs of hyperparathyroidism in CKD patients. [19]. A high prevalence of high turnover bone disease was noted in previous reports [20]. According to more recent reports, the most common condition in CKD stage 5 patients right before starting dialysis is low-turnover bone disease. [21]. However, there is evidence of skeletal resistance to parathyroid hormone (PTH) in patients with CKD-MBD, and the bone response to PTH varies. Consistent with Saito et al., 2004 [22], there was no statistically significant difference between smoking and hs CRP levels in CKD-MBD. One of the main concerns and a significant contributor to morbidity and mortality in dialysis patients is mineral bone disease secondary to chronic kidney disease. According to the kidney disease outcome quality initiative (KDOQI) guidelines, the overall prevalence of hyperparathyroidism is 34.3%, with incidences in the US, UK, and Japan being 47.6%, 33.1%, and 19.9%, respectively.[23] Although the maximum number of patients—19 patients, or 31.67% of the total—were in the 50–69 age range, indicating that the incidence of CKD rises with age, this difference was not statistically significant when compared to the mean occurrence of MBD in other study groups [24]. In their individual studies, Nakai and Kumchev et al. also demonstrated that there is no relationship between a patient's age and MBD. 32 (53.33%) of the patients in this study had CKD that had been present for one to three years, and there was no statistically significant correlation between the length of CKD and MBD [25]. Balon Pecovnic et al. In their individual investigations, Gabay et al. and Torres et al. also came to the same conclusions. Thirty-four of the 40 CKD patients who received hemodialysis in our study had MBD, while six did not. Six patients did not have MBD, while 14 patients with CKD who had never received hemodialysis did. There was no statistically significant correlation between MBD and hemodialysis, as indicated by values of $\chi 2 = 1.87$ and $P = 0.05$ in patients who never had hemodialysis as opposed to those who did. In their research, Ritz et al. found no qualitative differences between the bone findings of dialysis patients and those with stable advanced uremia. In our study, hyperparathyroidism was present in 95% of CKD patients, of whom 82.4% had MBD. Despite having normal serum iPTH, one patient (33.33%) had MBD. The serum iPTH test's sensitivity for early MBD detection in our study was 97.92%, which was highly significant, but its specificity was only 16.67%. Serum iPTH is employed as a surrogate biomarker in clinical settings to forecast. Serum iPTH is employed in clinical settings as a stand-in biomarker for bone turnover prediction. Nevertheless, research assessing the predictive power of intact PTH serum concentration for low- and high-turnover bone diseases has been underwhelming. The concentration of intact PTH generally raises the risk of high-turnover bone disease.[26] However, until intact PTH levels of 450–500 pg/mL are attained, it is difficult to predict with any degree of accuracy whether high-turnover bone disease will be present. For the prediction of low-turnover bone disease, intact PTH levels <100 pg/Ml are reasonably reliable [27], though they are not flawless. As we found in our research, elevated iPTH was linked to MBD. These studies serve as the primary basis for the K/DOQI guidelines, which suggest a

target intact PTH level of 150–300 pg/mL. [28] According to Chonchol et al., serum PTH levels gradually rise as kidney function declines. Serum levels of calcium and phosphorus that are within normal ranges are typically linked to this increase in PTH. Elevations of PTH were found in patients with early kidney disease. [29 According to Arici et al., secondary HPT arises early in the course of CKD and affects the majority of patients. Hemoglobin, serum creatinine, serum uric acid, serum phosphorus, serum calcium, serum ALP, and MBD did not significantly correlate in the current study group; the P value was 0.05. [30] Additionally, Mondry et al. and Barnas et al. found no statistically significant association between MBD and serum creatinine. [31] Finally, we discussed some of the study's limitations. The wide 95% CI indicates that the specificity of iPTH was estimated with poor precision due to the small sample size.[32, 33] We were unable to diagnose renal osteodystrophy by histophotometry because of the invasiveness of the bone biopsy test and the absence of consent [34]. One of the progressive conditions that results in an irreversible reduction in the glomerular filtration rate is chronic renal failure, which raises blood urea nitrogen and serum creatinine levels. [35] Chronic renal failure is most commonly caused by autoimmune diseases, diabetes, hypertension, and other conditions. because it is irreversible and gradually worsens, resulting in a 5–10% decrease in glomerular filtration rate when uremia levels are high.[36] The signs and symptoms of the disease are reflected in these blood biochemical alterations. Overt symptoms of uremia disappear as a result of chronic dialysis, which reduces the frequency and severity of these abnormalities. We sought to determine whether there were any differences between the serum electrolytes (potassium and sodium) before and after dialysis. The predialysis group had higher serum potassium levels than the postdialysis group (P-value 0.03 highly significant). [37] This study combines a novel non-invasive technique with biological indicators of bone and phosphate/calcium metabolism. According to a recent study, when renal function deteriorates, serum osteocalcin levels rise [38]. This study found that even moderate renal dysfunction (mean GFR 33 ± 12; maximal GFR 70 mL/min per 1.73 m2) was associated with the accumulation of serum osteocalcin during CKD. [39]

Conclusion:

Patients with chronic kidney disease benefit from this study when they have bone mineral disease. By using non-invasive techniques to estimate serum iPTH, osteocalcin, and (TRACP) 5b levels, bone mineral disease in patients with chronic kidney disease can be identified early and used as a prognostic biomarker.

Additional Information:

Disclosures:

Human subjects: Consent for treatment and open access publication was obtained or waived by all participants in this study. **Ethics statement:** Institutional ethics committee was convened in Pacific Medical College and Hospital, Udaipur, (Rajasthan) India. Ethical approval for the project approved by Institutional ethics committee, Pacific Medical College and Hospital, Udaipur, Rajasthan India. Informed written patient consent form for treatment and publication in open access journal has been obtained from each study participant prior to enrollment in study and sample collection. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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