Systemic Assessments Utilizing Saliva: Part 2 Osteoporosis and Use of Saliva to Measure Bone Turnover

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In this 2-part review series, the current uses of saliva as a diagnostic fluid are reviewed. Part 1 focused on known measurements of systemic conditions. In Part 2, the role of saliva to measure bone turnover with a special emphasis on osteoporosis are discussed. *Int J Prosthodont 2006;19:53–60.*

Osteoporosis has been defined by the World Health Organization as "a systemic skeletal disorder characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture."[1] Clinically, osteoporosis frequently manifests as a low to moderate trauma-induced fracture with the hip, spine (thoracolumbar vertebrae), and wrist most commonly affected.[2–4] Compromised muscular function and weakened bone structure occur in women after menopause and in men with advancing age. Peak bone mass is typically attained in young adulthood (early 30s) and, at least in women, remains fairly constant until the onset of estrogen deficiency subsequent to menopause.[5] The loss of the bone-protective effect of estrogen results in a biphasic loss of bone.[6,7] A rapid phase occurs in the first decade postmenopause and accounts for a 20% to 30% loss of trabecular bone and a 5% to 10% loss of cortical bone.[8] A second slow, continuous phase also occurs for the duration of a woman's life that accounts for an additional 20% to 30% loss of trabecular and cortical bone.[8] Increased bone remodeling (turnover) characterizes this bone loss, with a significant elevation in the rate of resorption outpacing a simultaneous but slower increase in bone formation. As a result, more and deeper resorption sites occur, which weaken trabeculae.[9] Persistent high bone turnover compromises trabecular thickness and integrity, leading to trabecular fenestrations that culminate in reduced bone strength.[9]

At present, there is an approximately 1 in 6 chance that a woman will experience an osteoporotic hip fracture in her lifetime compared to a 1 in 9 chance that she will suffer from breast cancer.[3,10] As the world's population ages, it is expected that disability pursuant to osteoporotic fractures will impose a greater burden on medical care costs and exacerbate loss of economic productivity in many regions of the world.[10] Already, the frequency of hip fractures is increasing by 1% to 3% per year in most areas of the world.[10,11] Osteoporosis is responsible for more than 1.5 million fractures annually in the United States, and the cost of fractures could be as high as US $20 billion annually, with a projected 3- to 8-fold rise by the year 2040.[12,13]

Osteoporosis can be characterized as a disease that is more prevalent in women, and being female can be considered a predictor of low bone mass. Other predictors include advancing age, gonadal hormone (estrogen or testosterone) deficiency, low body weight, low body mass index, family history of osteoporosis, nutritional deficiency, low calcium intake, smoking, excessive alcohol intake, low level of physical activity, chronic glucocorticoid use, and history of fracture.[2,3] In turn, the clinical risk factors for fractures are low bone mass, a history of falls, impaired cognition, low physical function, long hip axis, chronic glucocorticoid use, presence of an existing fracture, advancing age, and chronic use of various seizure medications (reviewed by Jordan and Cooper[2]). Green and coworkers recently reviewed the accuracy and precision of physical examination findings for the diagnosis of osteopenia, osteoporosis, or spinal fracture.[14] The authors concluded that no

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single maneuver is sufficient to rule in or rule out osteoporosis or spinal fracture. However, certain factors yielded the greatest positive likelihood ratios (LRs): weight less than 51 kg, LR +3.4 (95% confidence interval [CI] 5.0 to 10.8); tooth count less than 20, LR +3.4 (95% CI 1.4 to 8.0); rib-pelvis distance less than 2 fingerbreadths, LR +3.8 (95% CI 2.9 to 5.1); wall-occiput distance greater than 0 cm, LR +4.6 (95% CI 2.9 to 7.3); and self-reported humped back, LR +3.0 (95% CI 2.2 to 4.1).

World Health Organization data reveals that in the United States, 13% to 18% of women older than 50 years have osteoporosis and that another 37% to 50% have osteopenia (Table 1).[1] The National Osteoporosis Foundation in the United States estimates that half of white women and 1 in 4 white men older than 50 years will sustain at least one osteoporosis-related fracture in their remaining lifetime.[15] However, all ethnic groups in the United States are affected by osteoporosis. Five percent of non-Hispanic black women over age 50 are estimated to have osteoporosis; an estimated additional 35% have low bone mass, which puts them at risk of developing osteoporosis. Ten percent of U.S. Hispanic women aged 50 and older are estimated to have osteoporosis, and 49% are estimated to have low bone mass.[15]

The consequence of osteoporosis that has received the most attention is hip fracture, because it accounts for the highest mortality, morbidity, and economic cost.[16] Despite awareness that osteoporosis and related fractures will have a greater impact in all countries in the decades ahead, there is relatively little information to estimate current worldwide prevalence associated with hip fracture.[10,12,17] In general, however, the highest prevalence of hip fractures occurs in developed countries, specifically Scandinavia. While acknowledging disparities in reporting methods, Johnell and Kanis calculated variations in hip fracture probabilities for an array of countries for which meaningful data were available.[18] The authors set the probability of hip fracture in Sweden (reference population) to a value of 1.00 and compared other countries to this internal standard. Norway (1.24) and Iceland (1.02) had higher rates than Sweden, followed by Denmark (0.85), United States (0.78), China (Taiwan) (0.72), Germany (0.72), Switzerland (0.71), Finland (0.68), Greece (0.66), Canada (0.65), Netherlands (0.64), Singapore (0.62), Italy (0.61), United Kingdom (0.60), Australia (0.57), Portugal (0.57), France (0.41), Japan (0.39), Argentina (0.36), China (0.29), Turkey (0.18), Korea (0.18), Venezuela (0.17), and Chile (0.08). Following on, the authors categorized countries into very high risk (Norway to United States), high risk (China [Taiwan] to Portugal), medium risk (France to China), and low risk (Turkey to Chile). However, it is likely that the incidence of osteoporosis-related fractures will increase at a more rapid rate in Asia than other regions of the world.

Assessment and Prediction of Osteoporosis

Accurate measurement of bone strength is the key requirement to determine fracture risk.[19–22] However, determining bone strength relative to fracture resistance is necessarily assessed by surrogate markers, of which the most commonly used is bone mineral density (BMD).[23] BMD is a 2-dimensional, area projection measurement defined as the average concentration of mineral per unit area, expressed in grams per square centimeter. It is most often measured using dual-energy x-ray absorptiometery (DEXA). Low BMD is one of the strongest risk factors for fracture, and 75% to 90% of the variance in bone strength is related to BMD, even though increases in BMD are not necessarily associated with decreased risk of fracture.[24] BMD is frequently reported as either a z score or a *t* score. The z score compares a patient's BMD with the mean value in age-matched normal individuals and is potentially a better measure for young adults and children, who have not yet reached peak bone mass. The t score compares a patient's BMD to the mean value in a healthy young reference population representing standard peak bone mass. Often, both scores are adjusted for race and gender. The World Health Organization has proposed that BMD and fracture be combined in a stratified definition of osteoporosis that results in 4 categories related specifically to *t* scores.[1] The categories are presented in Table 1.

However, it should be emphasized that BMD is but one surrogate marker of bone strength.[20,21,25,26] Improved imaging techniques will likely permit the development of more sophisticated measures. For example, Melton and coworkers measured a variety of structural parameters and evaluated their association as compared with standard hip BMD, with fracture risk in 213 postmenopausal women and 200 men over the age of 50.[20] More than 37% of the women and 27% of the men had experienced a moderate trauma fracture, and 23% and 36% of women and men, respectively, had a history of severe trauma fracture. The results suggest that customizing assessments of fracture risk and osteoporotic status may be desirable. BMD, specifically femoral neck BMD, and structural parameters were strongly correlated in women who had experienced a moderate trauma fracture. However, in men with moderate trauma fractures, the best predictive model included age, femoral neck section modulus, and intertrochanteric buckling ratio. Fractures resulting from severe trauma were best predicted by structural parameters (femoral neck buckling ratio in women; intertrochanteric buckling ratio in men). Consequently, measures other than BMD may be as good or better in predicting fracture risk.

Table 1 World Health Organization Classification System for Osteoporosis Based On BMD

Classification	Definition
Normal	A value for BMD that is not more than 1 standard deviation (SD) below the young adult mean value (*t* score = 0 to -1)
Osteopenia	A value for BMD that lies between 1 and 2.5 SDs below the young adult mean value (*t* score between -1 and -2.5)
Osteoporosis	A value for BMD that is more than 2.5 SDs below the young adult mean value (*t* score = > 2.5)
Severe osteoporosis	A value for BMD that is more than 2.5 SDs below the young adult mean value in the presence of 1 or more fragility fractures

BMD and structural parameters permit determination of bone integrity. However, the ability to pinpoint a patient's location along the continuum of bone remodeling, in response to a panoply of mechanical and chemical stimuli, is valuable. Remodeling of bone typically is assessed by measuring the level of bone-specific proteins, or their degradation products, in serum and/or urine. It is generally accepted that changes in bony architecture take time to manifest, whereas changes in bone turnover manifest more quickly, thereby helping clinicians and patients know whether therapies are having the desired effect. Clearly, in individuals at high risk for fracture, minimizing bone resorption and/or maximizing bone formation are wanted outcomes, and numerous pharmacologic agents are designed to address one or both of these sides of the homeostasis equation. In most situations, bone resorption and bone formation move in tandem. For example, an increase in resorption caused by estrogen deficiency will lead to an increase in osteoblast numbers as compensatory mechanisms are activated. Therefore, the biphasic nature of postmenopausal bone loss is associated with a sustained elevation in the level of remodeling and, consequently, a sustained elevation in bone turnover.

The Relationship Between BMD and Bone Turnover

At present, BMD is commonly measured by DEXA, while measurement of bone turnover occurs via serum and urine assays that indicate an individual's bone formation and bone resorption status (a detailed description of bone turnover markers is included in the next section). In general, studies evaluating the relationship between BMD, bone turnover, and fracture risk fall into one of two categories. In retrospective studies, BMD and bone marker levels are examined in patients who have a history of osteoporotic fracture. The summary of results from retrospective studies suggests that, for patients with a fracture, the rate of bone formation is decelerated as measured by osteocalcin changes, while levels of urinary pyridinoline (PYD) crosslink, a marker of resorption, are elevated.[27] However, whether these changes are the result of acute or long-term effects of fracture is difficult to assess. The issue of postfracture analysis of bone markers is confounded by the inability to determine whether measured levels are a cause or a result of fracture. Overall, retrospective studies are difficult to interpret and, therefore, of limited value in correlating BMD, bone turnover, and fracture.

In prospective studies, BMD and bone marker levels are monitored in individuals with no history of osteoporotic fracture to determine whether changes in BMD or bone turnover occurred before or after an osteoporotic fracture. Relatively large populations are needed to include a sufficient number of fracture events to permit statistical analysis. Although such studies are limited in number, some general trends have preliminarily emerged, indicating that changes in markers of bone turnover are predictive of fracture. Specifically for bone formation, increased levels of osteocalcin, Type I collagen propeptides, and bone alkaline phosphatase (BAP) are associated with a 36% increase in the risk for vertebral fracture and a 64% increase in the risk for peripheral fracture during a mean 5-year follow-up.[28] The associations were independent of BMD. Of these markers, however, the association of BAP with fracture was the only one that reached statistical significance.[28] In addition, Ross et al demonstrated that increased levels of BAP were predictive of vertebral and nonvertebral fracture risk in a group of women followed for a mean of 2.7 years.[29] However, conflicting data has been presented by Akesson et al,[30] and further research is clearly needed to amass a confidence-generating body of evidence. Bone resorption marker data in prospective studies provides compelling evidence for an association with fracture. Analysis of urinary markers demonstrates that free PYD, free deoxpyridinoline (DPD), C-terminal collagen telopeptide (CTX), and N-terminal collagen telopeptide levels are all associated with increased fracture, with different studies identifying specific

markers as predictive to differing degrees.[27] For example, high urinary free DPD levels are associated with a 2-fold higher risk of all nonspine fractures in 207 women after a 3.8-year follow-up, and the odds ratio was sustained after data were normalized for BMD and disability status.[31] Serum CTX levels are also associated with hip fracture risk, although the evidence is more compelling if sampling was conducted in controlled conditions.[32] Taken together, it appears that as long as sampling is performed in a standardized manner, bone resorption markers are predictive of hip, vertebral, and peripheral fractures.

The relationship between bone turnover and BMD has been investigated to determine whether each is merely a surrogate for the other or whether they are more powerful predictors of fracture when used in combination. Given that the 2 entities measure similar and yet slightly different facets of bone physiology, it is not surprising that data generally show that one should not be used a surrogate for the other. Furthermore, because fracture occurs as a result of a variety of factors, eg, bone formation, bone resorption, trabecular architecture, cortical thickness, and trabecular thickness, it is also not surprising that BMD and bone turnover combined offer an enhanced perspective on the risk for fracture.[27,33–35] It is possible that the weaknesses in sensitivity and specificity of one are mitigated by the strengths in sensitivity and specificity of the other. For example, one large prospective study showed that combining a bone resorption marker (urinary osteocalcin [OC]) and hip BMD measurement can detect women at very high risk of fracture, since women with both low hip BMD and high bone resorption had a 4- to 5-fold higher risk of fracture than the general population.[36]

Evidence from a series of clinical trials investigating the effects of either hormone replacement therapy or bisphosphonate therapy demonstrate that changes in biochemical markers of bone turnover precede changes in BMD. In essence, short-term changes in bone turnover marker levels are related to the long-term response in bone mass, with the expected increase or stabilization of BMD associated with a slowing of bone turnover. For example, Ravn et al[37] showed that bone turnover markers predicted a change in spine BMD greater than 0%, with a high positive predictive value (PPV) and specificity, and that there was a trend toward better performance with bone resorption markers. Therefore, a significant advantage of assessing bone turnover markers is that an individual's response to therapy can be ascertained relatively soon after initiating therapy, rather than having to wait for changes in BMD to become detectable. However, clinicians often must rely on patients to procure urine samples and return them for testing to assess response to therapy, since serum collection at home is clearly impractical and inadvisable for patients to perform. Unfortunately, proper compliance with the collection protocol for urine, the current alternative to serum, and proper handling of the sample (second morning void) once collected is not always achieved. Furthermore, there is greater variability in urine assessments of bone turnover markers than in serum, complicating interpretation. Identification of an alternative biofluid to urine that is easily collected and handled at home by patients would significantly facilitate the clinician's ability to determine a patient's short-term response to therapy. Whole human saliva may be such an alternative biofluid.

Markers of Bone Turnover

The continuum of coupled bone resorption and bone formation may be assessed by measuring levels of bone-associated proteins in serum and urine. In essence, these measurements offer a window through which one can monitor, at any given time, whether a patient's serum or urine contains high or low levels of bone turnover markers. Invariably, a single measurement of a single marker has minimal utility. However, assessing changes in levels of single or multiple markers over time, or assessing relative ratios of markers to one another, can shed light on an individual's overall bone status. When combined with BMD scores, levels of bone turnover markers can be used to predict fracture risk. In general, bone turnover markers can be placed into one of two categories: markers of bone formation and markers of bone turnover, with each marker offering a different degree of specificity for bone.

Markers of Bone Formation

Typically, markers of bone formation represent the products (direct or indirect) of osteoblast function during various episodes of osteoblast differentiation and bone formation. Each marker presents advantages and disadvantages as they relate to specificity to bone, ease of detection, pre-analytic stability, and availability of sensitive and specific assays for detection.

Bone alkaline phosphatase. BAP is an approximately 45-kDa protein found in a variety of tissues and is found membrane-bound on numerous cell types.[38] Therefore, the total alkaline phosphatase (AP) level in serum reflects constituent APs from different sources, with the most common sources being liver, intestine, placenta, and bone, with the former and the latter each contributing almost 50% of the AP activity noted in serum.[39] The isoforms for liver, kidney, and bone AP arise from 1 tissue-nonspecific gene product and differ as a result of varying posttranslational modification.[40] Bone formation has been assessed by either

total serum AP or bone-specific AP, with the latter easier to assess in recent years with the development of improved enzyme-linked immunosorbent assays (ELISAs) that have lower cross-reactivity with the other forms of AP.[39] Not surprisingly, the bone specificity of these types of ELISA is likely of greater value than those that measure total AP.

Osteocalcin. OC is a relatively small (circa 5 kDa), gamma-carboxylated protein that binds to hydroxyapatite.[41] As a product only of osteoblasts, odontoblasts, and chondrocytes, levels of OC in serum are potentially useful, as bone-specific OC constitutes a significant majority of total OC.[41,42] The function of OC appears to be related to regulation of the rate of osteoid mineralization, and it likely ensures that mineralization occurs at an ideal (not too fast, not too slow) rate. OC knockout mice possess increased cortical and trabecular thickness and their bones may also be more stable than wild-type mice, indicating that OC acts to negatively regulate mineralization.[43] As a tissue, hypomineralized or hypermineralized bone may not offer optimal mechanical and physical properties, eg, flexure, fracture resistance, load-bearing capacity, and the role of OC may be to ensure that mineralization proceeds to promote optimal mineralization.

Measurement of serum OC levels is useful because OC is specific for bone formation.[44] However, interpretation of serum OC levels is challenging as a result of the presence of different circulating OC fragments. The fragments are products of serum degradation of intact OC or a result of release of fragments from resorbing bone.[45,46] The result is that immunoassays may be simultaneously yielding data relative to formation (osteoblast secretion of OC) and resorption (release of OC fragments from bone).[46] Fortunately, ELISAs utilizing 2-site sandwich immunoassay, which makes use of antibodies recognizing N-terminal and C-terminal epitopes to detect intact OC, are available.

Procollagen Type I propeptides. Type I collagen is initially secreted as a propeptide (procollagen), which is later enzymatically cleaved.[47] During bone synthesis, osteoblasts secrete a form of pre-procollagen containing N- and C-terminal extension peptides (PINP and PICP, respectively) that are removed by enzymatic activity of the extracellular matrix.[47,48] As a result, PINP (75 kDa) and PICP (115 kDa) levels in serum are indicative of newly synthesized collagen, and since other tissues that generate PINP and PICP do so relatively slowly, total circulating levels of PINP and PICP are reflective of bone formation.[49] Of the 2 markers, levels of PINP is a more sensitive marker of bone formation in osteoporosis, and its levels correlate better with bone formation than levels of PICP. PINP appears to be the better surrogate marker of bone formation, and its good stability offers additional technical advantages over PICP.[33]

Markers of Bone Resorption

Typically, markers of bone resorption represent the products (direct or indirect) of osteoclast function during various episodes of bone resorption. As with markers of bone formation, each marker of resorption presents advantages and disadvantages as they relate to specificity for bone, ease of detection, pre-analytic stability, and availability of sensitive and specific assays for detection. However, in contrast to markers of bone formation, markers of bone resorption may be detected in urine and/or serum.

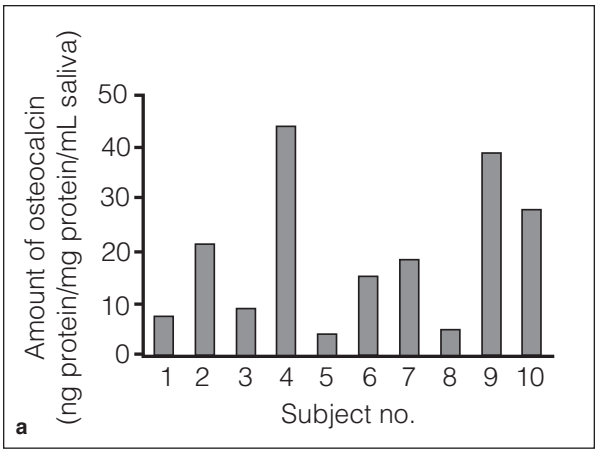
Hydroxypyridinium crosslinks of collagen. Hydroxypyridinium crosslinks of collagen form as a result of extracellular collagen maturation and fall into 2 principal categories: DPD and PYD. Their role is to add stability to collagen by bridging across collagen peptides, and they are incorporated into the final collagen molecule.[50,51] During bone resorption and the concurrent degradation of collagen, these crosslinks are released into serum and urine.[52] PYD and DPD are good sentinels of bone resorption because their serum/urinary levels are not associated with collagen formation and they are not affected by diet.[33,53,54] Furthermore, even though degradation of other tissues, such as cartilage and tendons, may contribute to the serum/urinary PYD and DPD levels, the turnover of these other tissues is far lower than that observed for bone, meaning that serum/urinary levels of these markers reflect bone resorption relatively well.[33,53] In recent years, specific ELISAs for PYD and DPD have been developed that demonstrate good specificity and reasonable sensitivity.

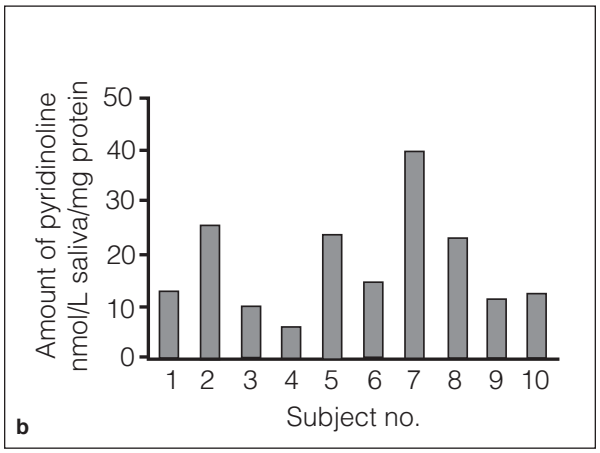
Crosslinked collagen telopeptides. The crosslinking of collagen, partially enabled by PYD and DPD, takes place at specific locations on the collagen molecule, specifically the N- and C-termini. Inasmuch as measurement of PYD and DPD reflects collagen degradation, the levels of N-terminus (NTX-1) and C-terminus (CTX-1) collagen peptides also reflect collagen degradation.[33,39,52]

Other markers of bone resorption. This topic has been reviewed recently by Seibel.[52,53]

Hydroxyproline. During synthesis of Type I collagen, proline residues, which are abundant in collagen, are hydroxylated. Urine contains hydroxyproline (Hyp) as a result of the degradation of bone collagen by the liver, and urine Hyp levels can be assayed to estimate bone resorption.[55] Unfortunately, urine Hyp may also have originated from degradation of newly synthesized collagen or from degradation of collagen from other tissues.[55] As a result, urinary Hyp provides relatively nonspecific assessment of bone resorption.

Bone sialoprotein. Bone sialoprotein constitutes 5% to 10% of the noncollagen organic component of bone and is a product of osteoblasts and odontoblasts.[56,57]





Figs 1a and 1b Concentrations of biochemical markers of bone turnover in unstimulated whole saliva of young adult women. **(a)** Osteocalcin units are ng protein/mL saliva/mg protein. **(b)** Pyridinoline units are nmol/L saliva/mg protein.

The function of bone sialoprotein appears to be in cell-matrix adhesion and in the supramolecular organization of mineralized tissue extracellular matrix.[56,57]

Tartrate-resistant acid phosphatase. Tartrate-resistant acid phosphatase (TRAP) is occasionally used to assess bone resorption, as the 5b isoform correlates to osteoclast activity.[58] TRAP is released by osteoclasts into the "sealed zone," in which the highly acidic environment promotes the solubilization of bone.[58] In measuring levels of TRAP, the goal is to assess bone resorption indirectly by directly assessing osteoclast activity.[59]

Bone Turnover Markers in Saliva?

The collection of serum and urine is cumbersome and invasive, and as a result, optimal assessment of bone turnover is inhibited. Monitoring systemic conditions by testing for the level of hormones, proteins, viral particles, or antibodies as described in Part 1[60] has led to investigation of whether saliva can be used to measure bone turnover. McGehee and Johnson used commercially available ELISAs to test for the presence of OC and PYD in the whole human saliva of 37 women.[61] Levels of OC and PYD in saliva correlated reasonably well with calcaneus BMD/t scores, suggesting that saliva may be a valuable tool for assessing human markers of bone turnover. In parallel work conducted by the present authors, the ability to detect OC and PYD in whole unstimulated human saliva was confirmed (Figs 1a and 1b). Briefly, saliva was centrifuged to pellet insoluble material, and clarified saliva samples were aliquotted and frozen. In 2 separate groups of 10 healthy female subjects between 20 and 23 years of age, OC and PYD were detected using commercially available non-radioactive ELISAs (Quidel Corporation) in the saliva of all 10 subjects. The mean normalized concentration of OC was 19.18 ng/mL saliva/mg protein (range 4.5 to 44.2). The mean normalized concentration of PYD was 16.94 nmol/L saliva/mg protein (range 4.9 to 36.7). Therefore, the possibility exists that saliva, an easily accessible and convenient biofluid, can be used in conjunction with, or in lieu of, serum and urine measures to assess bone turnover. Further research is necessary to determine whether salivary levels of bone turnover markers correlate with serum and/or urine measures. Given the interactive relationship between bone turnover, BMD, and fracture risk, these intriguing data necessitate research to define the role of saliva in assessing bone turnover.

Advantages and Limitations of Saliva as a Diagnostic Fluid to Measure Bone Turnover

The use of saliva as a diagnostic fluid may have certain limitations specifically related to the measurement of proteins. First, the potential for blood contamination must be considered when assessing the levels of bone turnover markers that are normally found circulating in serum. The majority of research measuring salivary levels of different proteins has not accounted for this potential source of error. Therefore, when collecting saliva (or any protein) as a surrogate for serum, measures aimed at limiting the potential for blood contamination should be employed, because many procedures and conditions can contaminate saliva. These include *(1)* history (within the past 6 weeks) of intraoral surgery, eg, periodontal surgery, endodontic surgery, oral surgery including exodontia, implant osteotomy; *(2)* history (within the past 2 weeks) of dental prophylaxis or any other dental procedure (eg, gingival retraction cord placement) that may have elicited intra-

oral bleeding; *(3)* toothbrushing or flossing within the past hour; *(4)* blood disorders associated with enhanced bleeding; and *(5)* history of desquamative gingivitis, pemphigus, pemphigoid, or erosive lichen planus. Recent evidence indicates that transferrin peroxidase is the best indicator of salivary blood contamination.[62] In general, it has been proposed that saliva samples with transferrin values above 1.0 μg/L be excluded from analysis, because their blood contamination level is moderate, and that samples with values above 0.5 μg/L should be excluded when testosterone is being measured. A second potential limitation relates to nonserum sources of bone turnover markers. The most likely source of bone markers in saliva that are not purely salivary in nature is gingival crevicular fluid (GCF). Bone turnover markers are detectable in GCF from diseased periodontal sites and are measured to assess local bone remodeling processes. However, the effect of GCF markers on salivary levels would be appear to be low; Bullon et al demonstrated that whereas GCF OC levels were higher in periodontitis sufferers than in nonperiodontitis individuals, salivary and serum OC levels were not correlated to periodontitis status.[63] Following on, it is possible that certain proteins may have intraoral bacterial analogues that can lead to erroneous salivary readings. For example, certain intraoral bacterial species produce AP. Consequently, ELISA systems, such as that used to generate the data shown in Fig 1, that do not crossreact with bacterial AP to a significant degree (< 2% crossreactivity) should be used.

Cortisol, a marker of stress, is frequently measured in saliva (discussed in Part 1 of this series[60]). However, cortisol measurements display diurnal variability, and to date, no literature has been presented regarding diurnal variability of bone turnover markers in saliva. Future research should be dedicated to determining whether it is necessary to standardize an individual's saliva collection appointment to a narrow time window to minimize any effect of diurnal variability.

Summary

Salivary diagnostics is a burgeoning field of interest as it offers clinicians, researchers, and patients the opportunity to learn about oral and systemic conditions by donating an easy-to-collect fluid. With an aging population that is less able to present routinely for appointments and in whom needlestick procedures may not always be feasible, the simple procurement methods for saliva make investigation of saliva worthwhile. Specifically with regard to bone turnover, preliminary evidence indicates that markers of bone turnover are detectable in whole saliva. Since there appears to be little correlation in bone turnover marker levels between GCF and saliva regardless of periodontitis status, data collected so far is promising in that salivary bone turnover marker levels may be indicative of systemic status.

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