Chronic Renal Failure andAluminum Toxicity on Dentin in Albino Rats

Azza El-Badry, Amel Radwan El-Hak and Nancy M. S. Abd el-Hay

Oral Biology, Faculty of Dentistry, Alexandria University, Egypt

Abstract: The objective of the present study was to examine the manifestations of uremia and aluminum loading on dentin structure and to determine whether characteristic changes, analogous to those seen in bone, occurs in the dentin of teeth in patients suffering from chronic renal failure (CRF) and may therefore be useful to the clinician in diagnosing renal osteodystrophy (ROD). Thirty albino rats with average 200g were randomized into 3 groups; control, study group I and II. CRF was induced in the study groups I&II by intraperitoneal injection of cisplatin 5mg/kg (BW) initially and then with two maintenance doses of 2.5 mg/kg BW every two weeks over a period of one month. Aluminum sulphate was given to the study group II by intraperitoneal injection 1 mg/200 g (BW) for 2 weeks, 3 times per week. After 2 months, all animals were sacrificed at the same time and the mandibles were isolated. Each mandible prepared for histological examination and atomic absorption spectrophotometric analyses. Strong evidence to support that uremia and aluminum deposition in rat's results in dentin changes reminiscent of those noted in bone was observed.

[Azza El-Badry, Amel Radwan El-Hak and Nancy M. S. Abd el-Hay. **Chronic Renal Failure and Aluminum Toxicity on Dentin in Albino Rats.** *J Am Sci* 2014;10(2):73-82]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 13

Keywords: Dentin, Bone, Chronic renal failure, aluminum toxicity, histological investigations.

Introduction

Chronic renal failure progresses slowly and is usually permanent. It is defined as the progressive and usually irreversible decline of the GFR, leading to azotemia.[5] CRF is the end result of a variety of renal diseases and is the major cause of death from renal disease. In the United States, diabetic nephropathy is the most common and hypertension is the second most common cause. Along with glomerulonephritis, these cause approximately 75% of all adult cases. In certain geographic areas, HIV-related renal disease is becoming common.[2,3] Less common causes are reflux nephropathy, kidney stones, prostate disease and autoimmune diseases.[4-6]Notably, the incidence and prevalence of CKD have shown a dramatic increase over the past two decades.[7] There are an estimated 920,000 patients on dialysis throughout the world, and this figure is growing by approximately 7-9% per year.[8]

The major systemic manifestations of CRF and uremia are fluid and electrolytes disturbances include dehydration, edema, hyperkalemia, and metabolic acidosis. Calcium, phosphate, and bone alterations include hyperphosphataemia, hypocalcemia, secondary hyperparathyroidism, and renal osteodystrophy. ROD is collective term describing the mixture of pathophysiological conditions that afflict the skeletal system of patients with CKD.[9] ROD is most serious in children because their bones are still growing. The spectrum of skeletal abnormalities seen in ROD is classified according to the state of bone turnover: High turnover bone disease or osteitisfibrosacystica represents the manifestation of hyperparathyroidism, characterized by increased osteoblasts and osteoclasts activity and peri-trabecular fibrosis. Low turnover bone disease includes: (i) Osteomalacia, characterized by a reduction in the number of osteoblasts and osteoclasts with an increase in the amount of osteoid. (ii) Adynamic bone disease, Characterized by marked decrease in osteoblasts and osteoclasts with normal or reduced osteoid. [82] Combinations of these abnormalities are called mixed ROD.[9] Within the past two decades, the prevalence of high turnover ROD has decreased while low bone turnover has become increasingly recognized. This trend is likely to reflect changes in the treatment of ROD and dialysis techniques.[10]

Chronic aluminum exposure and toxicity related to aluminum absorption and contaminated dialysis fluid continue to be a problem for many patients with CRF, particularly in South America and in some developing countries.[11]

The two most prevalent sources of aluminum in this population are water used to prepare dialysate and aluminum-containing phosphate binders.[12] Studies indicate that aluminum has a direct effect, inhibiting bone formation and resorption. There is also evidence for an indirect effect through the action of aluminum on parathyroid hormone synthesis and by its modulation of calcium activity.[11]

Bone biopsy is the gold-standard for the diagnosis of ROD.[13-15] It is done under local anesthesia and involves removing a small sample of bone from the hip and analyzing it by microscope.[15] However, due to its invasive nature, bone biopsies are not performed in clinical practice; traditional

constraints continue to be perceived because of the procedure's invasiveness and cost.[10] Serum aluminum levels are of limited value except as measurements of immediately previous exposure as it reflects the amount of the metal ingested over a short time frame, and do not reflect accurately the aluminum load in tissues.[16] Dentin is the mineralized tissue that forms the bulk of the tooth. During dentinogenesis, odontoblasts secrete unmineralized, collagen rich extracellular matrices termed predentin. As a precursor of dentin (a bone-like mineralized tissue), predentin lies between the mineralization front and the odontoblast layer. Later, the predentin is transformed to the mineralized tissue when apatite crystals are deposited within and around collagen fibrils.[17-19] This process requires mechanisms that control the site and rate of apatite formation. In other words, the rate of formation of the unmineralized precursor layer should be the same as that of mineralization. Imbalances of these dynamic processes would lead to pathological conditions such as expansion of the predentin layer and reduction in the dentin layer as observed in some human dentin diseases.[20] Several growth factors play a key role in physiological odontoblast differentiation. Considerable focus has been placed on those growth factors, particularly of the Transforming Growth Factor-beta (TGF-β) family, which may be directly involved in signaling cytodifferentiation of odontoblasts and odontoblast-like cells.[21] While proof of which growth factors are responsible for signaling of odontoblast differentiation in vivo is lacking, Transforming Growth Factor β1 (TGF-β1), TGF-β3, Bone Morphogenetic Protein-2 (BMP-2), and Insulin-like Growth factor-1 (IGF-1) appear capable of signaling odontoblast differentiation *in vitro*.[22]

Evidence has been reported concerning the important role of epigenetic factors in the control of odontoblast differentiation. The term "epigenetic" generally encompasses all the complex gene environment interactions responsible for phenotypic changes, i.e. cell competence. The gene "reelin" has been identified as characteristic of the odontoblast phenotype which is highly expressed in the brain, and has been suggested to play a role in dentin-pulp complex innervations.[23]

Odontoblasts are terminally differentiated ectomesenchymal cells that synthesize several collagenous and non-collagenous proteins. Components of the predentin matrix are synthesized first and then the specialized macromolecules necessary for dentin mineralization are synthesized.[24] In humans, there are 27 different types of collagen, expressed from 42 different collagen genes.[25] Type I collagen constitutes 85–90% of the dentin organic matrix,[26] and is the major protein in bone. The triple-helical (3D) structure of collagen was determined by fiber diffraction over 50 years ago,[27] and its many post-translational modifications have been characterized.[28] The abundance of collagen in bone and dentin and the elaborate biochemistry involved in its synthesis are evident in the diverse etiology and clinical manifestations of inherited defects involving both bone and dentin.[29] Histologically, two patterns of dentin mineralization can be observed, globular and linear calcification that seem to depend on the rate of dentin formation. Globular (or calcospheric) calcification involves the deposition of crystals in several discrete areas of matrix by heterogeneous capture in collagen. With continuous crystal growth, globular masses are formed that continue to enlarge and eventually fuse to form a single calcified mass. These spherical foci of mineralizing dentin are also termed globular dentin.[30] Mineralization of dentin is initiated at the predentin-dentin interface and forms the mineralization front. During globular mineralization the interface appears irregular, showing numerous rounded profiles as opposed to the smooth profile of normal dentin.[30] A lack of proper coalescence of calcospherites in the dentin results in an irregular mineralization front, and scalloped dentin.[20] Interglobular dentin is the term used to describe areas of unmineralized dentin where calcospherites have failed to fuse into a homogeneous mass within mature dentin. These areas are especially prevalent in human teeth in which the person has had a deficiency in vitamin D or exposure to high levels of fluoride at the time of dentin formation.[17,30,31] Several studies for age estimation in living individuals have been published demonstrating various accuracy, precision and reliability of the dentinal biopsy specimens.[32,33] In addition, the dentin biopsy was used for scanning electron microscopy and microradiograph to investigate the structural changes of dentinal tubules in specimens obtained from both sensitive and insensitive radicular dentin.[34]

2.Materialsand Methods

Thirty albino rats with average weight 200 g were used in this study. They were randomly assigned to three treatment groups,10 rats each. (Control group) was intraperitoneally injected with 9% saline. (Study group I) Chronic renal failure group was intraperitoneally injected by cisplatin 5mg/kg body weigh initially and then with two maintenance dose of 2.5 mg/kg BW every two weeks over a period of one month.[244,245] (Study group II) Chronic renal failure group with aluminum toxicity was intraperitoneally injected by cisplatin 5mg/kg body weigh initially and then with two maintenance dose of 2.5 mg/kg BW every two weeks over a period of one month.[35,36] Aluminum toxicity was induced in the study group (II) by intraperitoneal injection of aluminum sulfate, 1

mg/200 g (BW) for 2 weeks, 3 times per week.[37] After 2 months, all animals were sacrificed at the same time and the mandibles were isolated. Each mandible was labeled, decalcified and prepared for histological examination and spectrophotometric analyses.

Histological Studies :[38,39]

The samples were immediately fixed in 10 % neutral formalin and left at least 24 hours before tissue processing, washed, tissues were then dehydrated in ascending series of alcohol. Clearing the specimen by xylene and tissue samples were embedded in paraffin (56-58°C), then labeled and left to harden overnight. Paraffin tissue blocks were sectioned using a microtome into (6-8μm) thick serial sections for hematoxyline and eosin (H&E), and trichrome staining and (2μm) thickness for toluidine blue stain. Sections were dehydrated, cleared, and then mounted. Stained and Sections were microscopically examined.

Spectrophotometric analysis [40]

In their elemental form, metals will absorb ultraviolet light when they are excited by heat. Each metal has a characteristic wavelength that will be absorbed. The instrument looks for a particular metal by focusing a beam of ultraviolet light at a specific wavelength through a flame and into a detector. The sample of interest is aspirated into the flame. If that metal is present in the sample, it will absorb some of the light, thus reducing its intensity. The instrument measures the change in intensity. A computer data system converts the change in intensity into an absorbance. In the present study analyses were done to determine the aluminum content in different samples of serum, bone and dentin. At time of biopsy, the samples were weighted and placed in an aluminum free container. Bone marrow was removed by washing with a jet of deionized water. After drying at room temperature the bones were ground in stainless mills, the powdered bone was then acid digested and samples were analyzed using atomic absorption spectrophotometery.[41] For preparation of dentin specimens, enamel was removed by rotatory bur and dentin was obtained and acid digested with the same manner for bone preparation.

3. Results

Histological Results

In sections from the control groups, two layers were demonstrated in dentin by staining with H&E and trichrome stain (Fig. 1). The wide outer layer, homogeneous in appearance, represented the calcified dentin. The narrow inner light staining zone was the predentin. At high power the odontoblasts was columnar or high columnar phenotypes, and were pressed together and arranged in a pseudostratified layer.

The dentin consisted of three distinct layers that differed in their appearance; a thick inner predentin layer with accentuated incremental basophilic lines; a wide patchy middle layer; and an outer zone, which was homogeneous like normal dentin (Figs. 2 & 3). The usual palisade-like monolayer of odontoblasts was replaced by atypical multilayered arrangements of cells with variable orientation toward the predentin. High power of the middle layer stained lighter than the dentin, more like the predentin and revealed increased numbers of interglobular dentin reflecting the incomplete fusion of a large number of calcospherites globules. Moreover, the mineralization front in the study groups was irregularly shaped with many scattered eosin-staining globules (Fig. 4). Sections stained with trichrome stain for collagen study (Fig. 5) showed that the dentin in the control group stained an intense blue green and appeared homogeneously distributed. In contrast, the dentin in the study group I revealed patches of light-staining green intermixed with unstained areas. The collagen in the study group II appeared more extensively affected, it stained predominantly lighter similar to the predentin collagen. The color change in the study groups would signify differences in the organic matrix as compared with the control. A reduction in the number and variation in the size of the dentinal tubules that were irregularly embedded within the disturbed dentin matrix was also observed in the study groups (Fig. 5C). Some sections from study group II displayed a thick predentin and poorly organized dentin matrix at the apical region, whereas the lateral region revealed an increased number of Howship's lacunae with large multinucleated odontoclasts (Figs. 6, 6A & 6B). Sections from both study groups displayed disturbed collagen production in the predentin and adjacent dentin and absence or disorganized odontoblasts with large nuclei and small amount of cytoplasm (Figs. 7 & 9). On both study groups there was a definite correlation between the histology of dentin and bone. Sections showing thick predentin, impaired mineralization, and diffuse calcifications, the bone histology revealed osteoid covering most of the bone surface and evidence of marrow fibrosis (Figs. 6, 6A). Irregular mineralization front was observed in both dentin and bone. This is all characteristic of renal osteodystrophy. Other changes observed in study groups was an increasing number of pulp stones, prominent engorgement of the blood vessels and vascular calcifications in the pulp as compared with the control group (Figs. 8, 9). Focal calcifications in alveolar bone were also noted. Moreover, in some sections from the study groups osteodentin like-tissue was visible adjacent to an abnormally wide predentin (Fig. 10). The formation of osteodentin began at the periphery of the pulp and gradually advanced towards

the predentin. The odontoblasts lining this matrix often appeared disorganized. Small areas of focal calcification in periodontal ligament and marrow cavity have been also observed in these sections. Prominent metachromasia was observed in the dentin of the control group and varying degree of metachromasia was observed in the study groups (Fig. 11). The staining of odontoblasts with the toluidine blue varied from shades from dark blue (abnormal cells) to light blue (normal cells). In sections from control group most of the odontoblasts appeared light, and the fine cell structure such as the cell membrane and cytoplasm. In sections from the study groups, most of the odontoblasts displayed dark staining; these cells have large hyperchromatic nuclei varying in size and shape (Fig.13). The ratio of nucleus size to cell size was markedly increased.

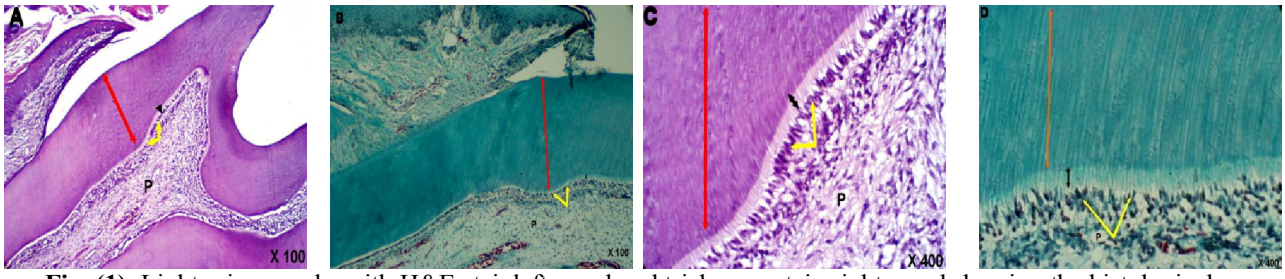

Fig. (1): Light micrographs with H&E stainleft panel and trichrome stain right panel showing the histological appearance of normal dentin. Two layers are evident, a wide outer layer, homogeneous in appearance representing the calcified dentin (red arrow), and a narrow inner light staining layer representing the predentin (black arrow). The odontoblasticlayer (yellow arrow) displays the usual palisade-like monolayer. P = pulp.

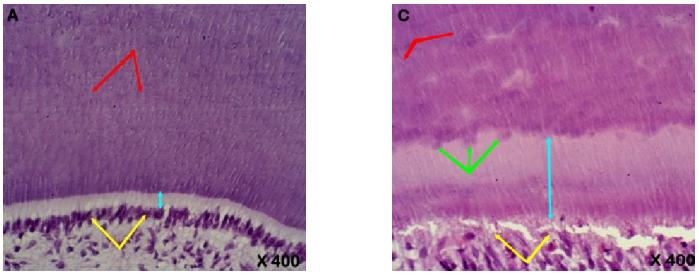

Fig. (2b): Light micrographs with H&E stain. Study group II (C) reveal a wide predentin (blue arrows) and irregular mineralization front with many scattered eosin-staining globules (green arrows) when compared with the control group (A). The dentinal tubulesappear sparse and irregularly shaped (red arrows), and most of the odontoblasts appear disorganized (yellow arrows) in the study groups.

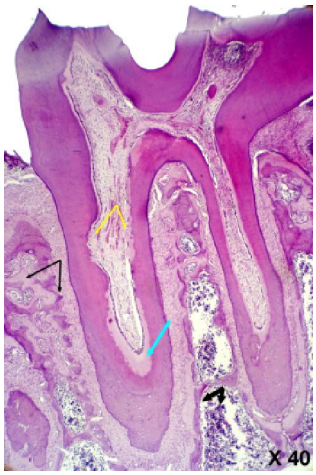

Fig. (3): Light micrograph with H&E stain from study group II shows prominent predentin at the apical region (blue arrow) and scalloping resorptive surfaces at the lateral regions (yellow arrows). The bone histology demonstrates prominent osteoid and tunneling bone resorption in some parts (thin black arrows), and thin osteoid with marrow fibrosis at other parts (thick black arrows).

Fig. (3a): Apical part magnified revealing thick predentin and poorly organized dentin matrix (green arrow). The bone histology reveals thick osteoid and tunneling cavity containing osteoclasts on the left(thin black arrows) and thin osteoid with accompanying marrow fibrosis on the right (thick black arrows).At high power (B) the irregular dentin-predentin interface with large globules is displayed (blue arrows).

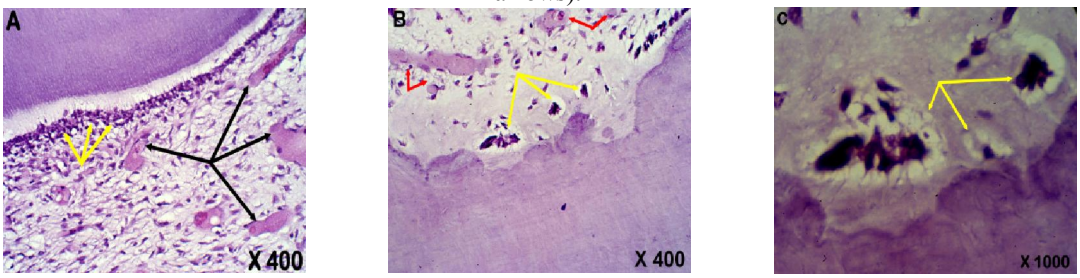

Fig. (3b): Note the prominent pulpal calcifications (Black arrows). Lateral part magnified revealingresorption lacunae withodontoclasts (yellow arrows). At high power (C) odontoclasts containing multiple nuclei are displayed.

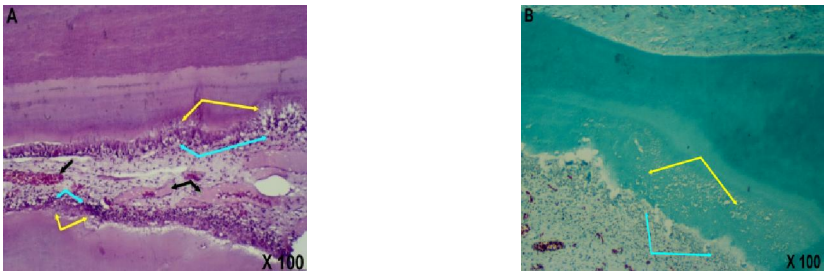

Fig. (4): Lightmicrographs with H&E stain A and trichrome stain B from the study group II. Large amounts of osteodentin-like matrix appear in the vicinity of the extremely wide predentin (yellow arrows). Note that the odontoblasts lining this matrix appear disorganized (blue arrows). The black arrows in A point to pulpal calcifications.

Spectrophotometric Analysis:

Comparison between the mean values and standard deviations of aluminum in serum, bone and dentin of the control and study groups were summarized in (tables 1,2,3 respectively), study group II was statistically higher than study group I or control group. On the other hand, Correlations between the control and the different study parameters (Table 5), showed a positive correlation between aluminum in bone and aluminum in serum..

Table (1): Aluminum in serum in control and study groups

Aluminum in serum	Control group	Study group I	Study group II
Range Mean S.D.	0.046 - 0.071 0.059 0.0068	0.11 - 0.19 0.145 0.0283	0.35 - 0.61 0.476 0.0687
U P1	6.22 0.0021*		
U P2	11.65 0.0001*		
U P3	6.98 0.001*		

* *P* is significant at *P*> 0.05.
*P***1** comparison between control and study group I.
*P***2** comparison between control and study group II.
*P***3** comparison between study group I and II.

Table (2): Aluminum in bone in control and study groups

Aluminum in bone	Control group	Study group I	Study group II
Range Mean S.D.	0.138 - 1.248 0.966 0.3280	4.21 - 5.813 5.195 0.5019	9.65 - 11.925 10.714 0.7463
U P1	13.98 0.00001*		
U P2	22.12 0.00001*		
U P3	8.23 0.0001*		

Table (3): Aluminum in dentin in control and study groups

Aluminum in dentin	Control group	Study group I	Study group II
Range Mean S.D.	1.121-1.672 1.319 0.1618	2.818-4.01 3.395 0.3894	4.491-11.55 7.697 2.2441
U P1	4.25 0.001*		
U P2	11.25 0.00001*		
U P3	5.18 0.006*		

Table (4): Correlations between different studied parameters

		Aluminum in serum	Aluminum in bone
Aluminum in bone	R	0.929(**)	
	P	0.000	
Aluminum in dentin	R	0.805(**)	0.870(**)
	P	0.000	0.000

r correlation coefficient.
* *P* is significant at *P*> 0.05.
** Correlation is significant at the 0.01 level (2-tailed)

4. Discussion

The kidney plays a critical role in the overall regulation of mineral. Renal osteodystrophy (ROD) in its broadest context encompasses all the disorders of bone and mineral metabolism caused by chronic renal failure (CRF). Prominent among these are disturbances in calcium, phosphorus and vitamin D metabolism that ultimately lead to alterations in parathyroid gland function and to various types of renal bone disease.[42,43]

The most prominent histological changes seen in most uremic animals were widening of the predentin layer, lack of proper coalescence of calcospherites, an irregular mineralization front, and large amount of interglobular dentin. In addition to this, the trichrome stained sections showed that the dentin in the control group stained an intense blue green and appeared homogeneously distributed. Whereas, in the study groups the dentin revealed patches of light-staining green intermixed with unstained areas or stained predominantly lighter similar to the predentin collagen, indicating differences in the organic matrix as compared with the normal layers.

An interesting property exhibited by toluidine blue is called metachromasia. Toluidine blue staining clearly demonstrated that there is an alteration in the formation of the organic matrix which may potentially interfere with the mineral nucleation and subsequent coalescence of calcospherites in forming a defined mineralization front. The failure of sections stained with toluidine blue to take up the purple staining of the control dentin is taken to indicate the decreased levels and less distribution of the most strongly acidic macromolecules in the dentin and predentin of the uremic rats relative to the control rats. Further, the zones of alternating intensity of metachromasia in dentin of study groups would signify an irregular calcification in contrast to control dentin.

The odontoblasts in the study groups were always disorganized, reflecting the irregular activity of odontoblasts. Equally interesting is that toluidine blue is a classic nuclear dye used for metachromatic and orthochromatic staining of chromatin. These features have been shown to be a sensitive structural probe for DNA secondary structure and packaging in situ. The dye is taken up by the nuclei of abnormal cells manifesting increased DNA synthesis and stains the cells dark blue, whereas, normal cells stain light blue.[44,45] Hence, it has been used for many years as an aid in the detection of cell abnormalities.[46]

The odontoblasts in the study groups appeared light, and the fine cell structure such as the cell membrane and cytoplasm was clearly seen. In contrast, in the study groups most of the odontoblasts displayed dark staining or different shades of blue with large hyperchromatic nuclei. Gradients of increasing nucleus-cytoplasm ratios were also observed. The proportion of dark cells with abnormal DNA conformation, detected by the toluidine blue in study group II was more prominent when compared with the study group I. These results would signify defects in the synthetic and/or secretory activity of odontoblasts.

A number of investigators have proposed multiple biological functions to DMP1 in odontogenesis, and osteogenesis. In one study, the investigators suggested that DMP1 acts as a hydroxyl-apatite nucleator and also controls cell differentiation through targeting the nucleus and/or interacting with cell-surface integrin/CD44 receptors.[47] In only one

study, it has been observed that exogenous DMP1 added to exposed dental pulp could act as a morphogen trigger and/or promoter of the differentiation of undifferentiated ectomesenchymal cells in the pulp toward the odontoblast lineage.[48]

In addition to its postulated function as a hydroxyapatite nucleator, DMP1 also is involved in calcium and phosphate metabolism through the kidney,[49] it has been implicated in the regulation of phosphate homeostasis through fibroblast growth factor 23.[50] Other investigators have shown that during postnatal development, Dmp1-null pups develop abnormalities, which are typically rickets and osteomalacia.[51-53]

Other changes in study groups was an increasing number of pulp stones, prominent engorgement of the blood vessels and vascular calcifications in the pulp as compared with the control. Elevated Ca x P product and hyperphosphataemia are viewed as important risk factors for cardiac and metastatic calcification.[12,54,55] High PTH itself induces increases in intracellular calcium and abnormal lipid metabolism that promote soft tissue calcifications.[56] Moreover, both severe hyperparathyroidism and marked hyperparathyroidism have been reported to promote soft tissue calcification in ESRD patients.[57-59] Another pathological change induced by uremia and aluminium toxicity in the present work was the deposition of osteodentin-like matrix. Osteodentin has also been observed after cavity preparation, dental pulp capping, and in subcutaneously transplanted teeth.[60-62] Moreover, it has been demonstrated that uremia significantly increases predentin width and induce deposition of large amounts of osteodentin-like matrix-containing cells in the pulp chamber.[63]

Spectrophotometric analysis demonstrated that the serum aluminum level and dentin aluminum content (tables 1&3) and were significantly increased in both study groups I and II when compared to control group. Aluminum is ubiquitous in the environment, making its avoidance difficult. it can enter the body through the diet owing to its natural presence in many foods, and from leaching into food by aluminum-containing cooking utensils.[64] It has been reported that healthy subjects with normal renal function are also at risk of long-term low-grade aluminum intoxication since they retain 4% of the aluminum consumed and might thus also accumulate aluminum and eventually be at risk of long-term low-grade aluminum intoxication that can affect tissue health.[65] All the above reports may help explain the increased dentin aluminum content in study group I as compared with the control one.

Although there was a significant difference between both study groups as regards the dentin aluminum content (Fig. 5), this difference did not seem to have a great impact on the histological results. This finding may be directly related to the short term, low dose aluminum administration in the study group II.

Induction of uremia and aluminum overload in rats showed dentin changes reminiscent of those noted in renal osteodystrophy. On both study groups it seems that there is a definite correlation between the mineralization status of bone and dentin. In sections revealing normal or thin predentin, and reduction in odontoblasts number, the bone histology demonstrated a thin or normal osteoid, and a paucity of cells along trabecular bone surfaces (Fig. 6). Whereas, sections showing thick predentin, impaired mineralization, and diffuse calcifications (Figs. 6, 6A, 6B), the bone histology revealed osteoid covering most of the bone surface and evidence of focal calcifications. Irregular mineralization front was observed in both dentin and bone. The histological results exhibited more histological signs of osteomalacia and fewer signs of adynamic bone disease. This finding is in agreement with the previous reports,[10,66] which found a relatively high prevalence of osteomalacia. Spectrophotometric analyses further confirmed that dentin changes can be quite similar to changes in bone, as a significant positive correlation between bone and dentin aluminum content which was illustrated in table (4).

Bone and dentin are both mineralized connective tissues sharing the main organic matrix constituents and the mineral phase. Therefore, systemic interference with bone formation has been shown to affect dentinogenesis.[67] Dentin defects have been found in human teeth from patients with renal transplants,[68] dentinogenesisimperfecta type III,[69], in hypophosphatemic vitamin D-resistant rickets,[70,71], and during disturbance of calcium and phosphate metabolism.[72-74]

The findings of the previous studies showed a wide spectrum of changes, ranging from mild disturbances with thicker predentin layer and increasing tubule irregularity, to widespread formation of dysplastic dentin.[75,76] In addition, it has been suggested that the dentin is the metabolic equivalent of osteoid in bone and that increases in the predentin thickness may therefore be a reliable indicator of osteomalacia and that the term odontomalacia might be used to describe the changes occurring in the dentin of these patients.[77] Others have reported that children or adults with poor renal function develop defects in primary and permanent dentition.[78,79]

To date, bone biopsy is the most informative diagnostic tool and provides information on the type of ROD, the degree of severity of the lesions, and the presence and amount of aluminum deposition in bone. Moreover, bone biopsies have been used for research

purposes to assess the effects of new therapies on bone.

The delicacy of the dentin reaction to the various experimentaldescribed conditions suggests the feasibility of using the dentin as a possible biomarker for ROD. Dentin is the product of odontoblasts exclusively and odontoblasts do not deposit extracellular matrices other than dentin. Furthermore, unlike bone, dentin does not undergo remodeling. In theory, dentin examination seems ideal and has been applied to detect alterations in mineralization caused by metabolic disturbances.[71,80,81] In addition, it has been shown that the effects of fluoride in the uremic rat incisor are primarily in the dentin.[63] Moreover, dentinogenesisimperfecta, an autosomal dominant disorder of the tooth primarily affects dentin biomineralization.[82-84]

References

1. Abdel Hamid MJ, Dummer II CD, Pinto LS: Systemic conditions, oral findings and dental management of chronic renal failure patients: general considerations and case report. Braz Dent J 2006; 17(2).
2. Proctor R, Kumar N, Stein A, Moles D, Porter S. Oral and dental aspects of chronic renal failure. J Dent Res 2005; 84: 199-208.
3. Perazella MA. Acute renal failure in HIV-infected patients: a brief review of common causes. Am J Med Sci 2000; 319(6): 385-91.
4. Greenwood M, Meechan JG, Bryant DG. General medicine and surgery for dental practitioners Part 8- Br Dent J 2003; 195: 181-4.
5. Rossi SS, Glick M. Dental Considerations for the patient with renal disease receiving hemodialysis. J Amer Dent Assoc 1996; 19: 127-211.
6. United States Renal Data System. Atlas of End Stage Renal Disease. United States Renal Data System. 2008.
7. Coresh J, AstorBC, Greene T, Eknoyan G, LeveyA S. Prevalence of chronic kidney disease and decreased kidney function in the adult US population: Third National Health and Nutrition Examination Survey, Am. J. Kidney Dis 2003; 41: 1-12.
8. Annual Data Report. United States Renal Data System, Minneapolis. 2007.
9. El-KishawiAb, El-NahasAM. Renal Osteodystrophy: Review of the Disease and its Treatment. Saudi J Kidney Dis Transpl 2006; 17: 373-82.
10. Martin KJ, Olgaard K, Coburn JW. Diagnosis, assessment, and treatment of bone turnover abnormalities in renal osteodystrophy. Am J Kidney Dis 2004; 43(3): 558-65.
11. Cannata-Andia JB, Fernandez-Martin JL. The clinical impact of aluminum overload in renal failure. Nephrol Dial Transplant 2002; 17(Suppl 2): 9.
12. Hruska KA, Saab G, Mathew S, Lund R. Renal osteodystrophy, phosphate homeostasis, and vascular calcification. Semin Dial. 2007; 20(4): 309-15.
13. Hutchison AJ, Whitehouse RW, Boulton HF. Correlation of bone histology with parathyroid hormone, vitamin D3, and radiology in end-stage renal disease. Kidney Int 1993; 44(5): 1071-7.
14. Ho LT, Sprague SM. Percutaneous bone biopsy in the diagnosis of renal osteodystrophy. SeminNephrol 2002; 22(3): 268-75.
15. National Kidney and Urologic Diseases Information Clearinghouse, National Institutes of Health. Last Editorial Review: 12/27/2007.
16. Goodmar WG, DuarteME. Aluminum effects on bone and role in the pathogenesis of renal osteodystrophy. Miner Electrolyte Metab 1991; 17: 221-32.
17. NanciA.Ten Cate's Oral Histology Development, Structure, and Function. 7th St. Louis: Mosby, 2008. 192-239.
18. Lu Y, Ye L, Yu S, Zhang S, Xie Y, McKee MD. Rescue of odontogenesis in Dmp1-deficient mice by targeted re-expression of DMP1 reveals roles for DMP1 in early odontogenesis and dentin apposition *in vivo*. DevBiol 2007; 303: 191-201.
19. Butler WT, Brunn JC, Qin C. Dentin extracellular matrix (ECM) proteins: comparison to bone ECM and contribution to dynamics of dentinogenesis. Connect Tissue Res 2003; 44: 171-8.
20. Levin LS, Leaf SH, Jelmini RJ, Rose JJ, Rosenbaum KN. Dentinogenesisimperfecta in the Brandywine isolate (DI type III): clinical, radiologic, and scanning electron microscopic studies of the dentition. Oral Surg oral Med oral Pathol 1983; 56, 267-74.
21. Ruch JV, Lesot H, Begue-Kirn C. Odontoblast differentiation. Int J DevBiol 1995; 39: 51-68.
22. Smith AJ, Lesot H. Induction and regulation of crown dentinogenesis: embryonic events as a template for dental tissue repair. Crit Rev Oral Biol Med 2001; 12: 425.
23. Lesot H, Lisi S, Peterkova R, Peterka M, Mitolo V, Ruch JV. Epigenetic signals during odontoblast differentiation. Adv Dent Res 2001; 15: 8-13.
24. Lisi S, Peterkova R, Peterka M, Vonesch JL, Ruch JV, Lesot H. Tooth morphogenesis and pattern of odontoblast differentiation. Connect Tissue Res 2003; 44(Suppl 1): 167-70.
25. Myllyharju J, Kivirikko KI. Collagens modifying enzymes and their mutations in humans, flies and worms. Trends Genet 2004; 20:33-43.
26. Linde A, Bhown M, Butler WT. Non collagenous proteins of dentin. A re-examination of proteins from rat incisor dentin utilizing techniques to avoid artifacts. J BiolChem 1980; 255: 5931-42.
27. Rich A, Crick FH. The structure of collagen. Nature 1955; 176: 915-6.
28. Viguet-Carrin S, Garnero P, Delmas PD. The role of collagen in bone strength. OsteoporosInt 2006; 17: 319-36.
29. Kim JW, Simmer JP. Hereditary Dentin Defects. Journal of Dental Research 2007; 86(5): 392-9.
30. Avery JK, Steele PF, Avery N. Oral Development and Histology. 3rd ed. Thieme, 2002. 172-89.
31. Berkovitz KB, Holland GR, Moxham BJ. Oral Anatomy, Histology and Embryology. 3rd ed. Mosby, 2002. 125-48.
32. Ontani S, Ohhira H, Watanabe A, Ogasawara A, Suimoto H. Estimation of age from teeth by amino acid

racemization: Influence of Fixative. J Forensic Sci 1997; 42(1): 137-9.
33. Herschaft E, Alder M, Ord D, Rawson R, Steven Smith E. Manual of Forensic Odontology. Am Soc Forensic Odontol 2007; 53-66.
34. Yoshiyama M, Masada J, Uchida A, Ishida H. Scanning Electron Microscopic Characterization of Sensitive vs. Insensitive Human Radicular Dentin. Journal of Dental Research. 1989; 68(11): 1498-502.
35. Mylones AL Massoulas GB, Nicolatou O, Dontas IA, Nakopoulou L, Stefanidis CJ. Progress of ossification and epithalization of wounds after simple or surgical extraction of teeth in rats with chronic renal failure an experimental study. J Oral MaxillofacSurg 2000; 38(1): 55-45.
36. Radwan el Hak A. The histological changes of teeth structures and supporting tissue due to chronic renal failure (in albino rats). Egy dent Journal 2001; 47: 1321: 7.
37. Smith AJ, Faugere MC, Abreo K, Fanti P, Julian B, Malluche HH. Aluminum related bone disease in mild and advanced renal falure: evidence for high prevalence and morbidity and studies an etiology and diagnosis. Am J Nephrol 1986; 6: 275-83.
38. Bashkar SN. Orban oral histology and embryology-11 reduction. St. Louis, Baltimore, Boston, Chicago, London, Philadelphia, Sydney, Torteno: Mosby, 1990. 365, 339-41, 349-50, 470-3.
39. Kelly JW. Staining of macromolecules: possible mechanisms and examples.Vol 84, No. 4: Pages 139-58.
40. Marie-claude, faugere, Malluche HH. Stainable aluminum and not aluminum content reflects bone histology in dialyzed patients, division of nephrology, university of kentucky, lexingren, kentucky, USA. Kidney Inter 1986; 30: 717-22.
41. Smeyers-Verbeke J, Verbeelen D. Determination of aluminum in bone by atomic absorption spectroscopy. ClinChem 1985; 31(7): 1172-4.
42. Goodman WG. Medical management of secondary hyperparathyroidism in chronic renal failure. Nephrol Dial Transplant 2003; 18(Suppl 3): iii2-8.
43. Goodman WG. Recent developments in the management of secondary hyperparathyroidism. Kidney Int 2001; 59: 1187-201.
44. Butikova J, Ivanov A, Pjanova D. Cytometry2003; Part A 52A:19-27.
45. Erenpreisa J, Freivalds T, Roach H, Alston R. Apoptotic cell nuclei favour aggregation and fluorescence quenching of DNA dyes. Histochem Cell Biol 1997;108:67-75.
46. Erenpreiss J, Jepson K, Giwercman A, Tsarev I, Erenpreisa Je, Spano M. Toluidine blue cytometry test for sperm DNA conformation: comparison with the flow cytometric sperm chromatin structure and TUNEL assays. Human Reproduction 2004; 19(10): 2277-82.
47. Gajjeraman S, Narayanan K, Hao J, Qin C, George A. Matrix macromolecules in hard tissues control the nucleation and hierarchical assembly of hydroxyapatite. J Biol Chem 2007; 282: 1193-204.
48. Narayanan K, Gajjeraman S, Ramachandran A, Hao J, George A. Dentin Matrix Protein 1 Regulates Dentin Sialophosphoprotein Gene Transcription during Early Odontoblast Differentiation. J BiolChem 2006; 281: 19064-71.
49. Terasawa M, Shimokawa R, Terashima T, Ohya K, Takagi Y, Shimokawa H. Expression of dentin matrix protein 1 (DMP1) in nonmineralized tissues. J Bone Miner Metab 2004; 22: 430-8.
50. Qin C, D'Souza R, Feng JQ. Dentin matrix protein 1 (DMP1): new and important roles for biomineralization and phosphate homeostasis. J Dent Res 2007; 86: 1134-41.
51. Feng JQ, Ward LM, Li S, Lu Y, Xie Y, Yuan B. Loss of DMP1 causes rickets and osteomalacia and identifies a role for osteocytes in mineral metabolism. Nat Genet 2006; 38: 1310-5.
52. Ling Y, Rios HF, Myers ER, Lu Y, Feng JQ, Boskey AL. DMP1 depletion decreases bone mineralization in vivo: an FTIR imaging analysis. J Bone Miner Res 2005; 20: 2169-77.
53. Ye L, Mishina Y, Chen D, Huang H, Dallas SL, Dallas MR. Dmp1-deficient mice display severe defects in cartilage formation responsible for a chondrodysplasia-like phenotype. J BiolChem 2005; 280: 6197-203.
54. Goodman WG, Goldin J, Kuizon BD, Yoon C, Gales B, Sider D, Wang Y, Chung J, Emerick A, Greaser L, Elashoff RM, Salusky IB. Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. N Engl J Med 2000; 342: 1478-83.
55. Raggi P. Detection and quantification of cardiovascular calcifications with electron beam tomography to estimate risk in hemodialysis patients. ClinNephrol 2000; 54: 325-33.
56. Cozzolino M, DussoAS, Slatopolsky E. Role of Calcium-phosphate product and bone-associated proteins on vascular calcification in renal failure. Am SocNephrol 2001; 12: 2511-6.
57. Rostand SG, Drueke TB. Parathyroid hormone, vitamin D, and cardiovascular disease in chronic renal failure. Kidney Int 1999; 56: 383-92.
58. Tsuchihashi K, Takizawa H, Torii T, Ikeda R. Hypoparathyroidism potentiates cardiovascular complications through disturbed calcium metabolism: Possible risk of vitamin D-3 analog administration in dialysis patients with end-stage renal disease. Nephron 2000; 84: 13-20.
59. Galassi A, Spiegel DM, Bellasi A, Block GA. Accelerated vascular calcification and relative hypoparathyroidism in incident haemodialysis diabetic patients receiving calcium binders. Nephrol Dial Transplant 2006; 21: 3215-22.
60. D'Souza RN, Bachman T, Baumgardner KR, Butler WT, Litz M. Characterization of cellular responses involved in reparative dentinogenesis in rat molars. J Dent Res 1995); 74: 702-9.
61. Hosoya A, Yoshiba K, Yoshiba N. An immunohistochemical study on hard tissue formation in a subcutaneously transplanted rat molar. Histochem Cell Biol 2003); 119: 27-35.

62. Andelin WE, Shabahang S, Wright K. Identification of hard tissue after experimental pulp capping using dentin sialoprotein (DSP) as a marker. J Endod 2003; 29: 646-50.
63. Lyaruu DM, Bronckers ALJ, Santos F, Mathias R, DenBesten P. The effect of fluoride on enamel and dentin formation in the uremic rat incisor. Pediatric Nephrology 2008, 4 May.
64. PriestND. The biological behaviour and bioavailability of aluminium in man, with special reference to studies employing aluminium-26 as a tracer: review and study update. J Environ Monit 2004; 6: 375-403.
65. Hellstrom HO, Mjorbeg B, Mallmin H, Michaelsson K. No association between the aluminium content of trabecular bone and bone density, mass or size of the proximal femur in elderly men and women. Osteoporosis International 2006;16(12):1982-8.
66. Shin SK, Kim DH, Kim HS. Renal osteodystrophy in pre-dialysis patients: ethnic difference? Perit Dial Int 1999; 2: S402-7.
67. Hietala E-L, Larmas M. Evidence that high-sucrose diet reduces dentin formation and disturbs mineralization in rat molars. J Dent Res 1995;74: 1899-1903.
68. Nasstrom K, Moller B, Petersson A. Effect on human teeth of renal transplantation: a postmortem study. Scand J Dent Res 1993; 101: 202-9.
69. Leaf LS, Jelmini SH, Rose RJ, Rosenbaum KN. Dentinogenesisimperfecta in the Brandywine isolate (DI type III): Clinical, radiologic, and scanning electron microscopic studies of the dentition. Oral Surg Oral Med Oral Pathol 1983; 56: 267-74.
70. Murayama T, Iwatsubo R, Akiyama S, Amano A, Morisaki I. Familial hypophosphatemic vitamin D-resistant rickets: dental findings and histologic study of teeth. Oral Surg Oral Med Oral Pathol Oral RadiolEndod 2000; 90: 310-6.
71. Boukpessi T, Septier D, Bagga S, Garabedian M, Goldberg M, Chaussain-Miller C. Dentin alteration of deciduous teeth in human hypophosphatemic rickets. Calcif Tissue Int 2006; 79: 294-300.
72. Seeto E, Seow WK. Scanning electron microscopic analysis of dentin in vitamin D-resistant rickets: assessment of mineralization and correlation with clinical findings. Pediatr Den1991;13(1):43-8.
73. Salusky IB. Bone and mineral metabolism in childhood end-stage renal disease. PediatrClin North Am 1995; 42: 1531-50.
74. Leonard MB, Zemel BS. Current concepts in pediatric bone disease. PediatrClin North Am 2002; 49:143-73.
75. Clark DB, Wysocki GP. Dentin in chronic renal failure: an ultrastructural study. J Oral Pathol 1988; 17: 60-9.
76. Lucas VS, Roberts GJ. Oro-dental health in children with chronic renal failure and after renal transplantation: a clinical review. PediatrNephrol 2005; 20: 1388-94.
77. Nasshem K, Molter B, Petersson A. Effect of human teeth of renal transplantation. A postmortem study. Scand J Dent Res 1993; 101(4): 202-9.
78. Al Nowaiser A, Roberts GJ, Trompeter RS, Wilson M, Lucas VS. Oral health in children with chronic renal failure. PediatrNephrol 2003; 18: 39-45.
79. Viljoen A, Singh DK, Twomey PJ, Farrington K. Analytical quality goals for parathyroid hormone based on biological variation. ClinChem Lab Med 2008; 46: 1438-42.
80. Ikeda Y, Iki M, Morita A, Aihara H, Kagamimori S, Kagawa Y, et al. JPOS Study Group. Age-Specific Values and Cutoff Levels for the Diagnosis of Osteoporosis in Quantitative Ultrasound Measurements at the Calcaneus with SAHARA in Healthy Japanese Women: Japanese Population-Based Osteoporosis (JPOS) Study. Calcif Tissue Int 2002;71: 1-9.
81. Shafer WG, Hine MK, Lay, BM. A textbook of oral pathology. 2nd ed. Philadelphia: W.B. Saunders company, 1974. 592-5.
82. Zhang X, Zhao J, Li C, Gao S, Qiu C, Liu P, et al. DSPP mutation in dentinogenesisimperfecta Shields type II. Nat Genet 2001); 27: 151-2.
83. Tracy WE, Steen JC, Steiner JE, Buist NRM. Analysis of dentine pathogenesis in vitamin D. Resistant Rickets. Oral Surg 1971; 32: 38-44.
84. Rajpar MH, Koch MJ, Davies RM, Mellody KT, Kielty CM, Dixon MJ. Mutation of the signal peptide region of the bicistronic gene DSPP affects translocation to the endoplasmic reticulum and results in defective dentine biomineralization. Hum Mol Genet2002; 11(21):2559-65.

2/8/2014