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The Role of pH in Patients Receiving Long Term Bisphosphonate Therapy

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Abstract:

Purpose: Bisphosphonate (BP)-associated osteonecrosis of the jaw (BON) lacks a defined pathophysiologic mechanism and treatment regimen. This current study was designed to be part of a parent BON pathogenesis series of protocols to investigate the contributing factors of causation. The following hypothesis was tested: Subjects receiving long term bisphosphonate therapy that have developed BON have a more acidic oral environment i.e. as measured by salivary pH than normal (no BP exposure) subjects and those receiving long term bisphosphonate therapy that have not developed BON. This study assessed the pH of saliva in subjects receiving long term bisphosphonate therapy so that possible oral buffers could eventually be developed and investigated as possible treatments i.e. to reduce the acidic effects BPs such as zoledronic acid (ZA) have in BON wound healing.

Methods**:** Using the fully IRB approved HIPAA partial waiver for recruitment, subjects were selected based on history and physical examination in order to document previous and/or current use of bisphosphonates. Standard of care physical examination was used to determine the presence or absence of BON

lesions intraorally. As part of a fully IRB approved protocol the informed consent process was used and for those willing to participate and having signed the consent document, we collected the single sample of saliva. We employed these ex-vivo studies using pH measurement on ~5 ml of fresh unstimulated whole saliva collected over a 5 minute time period at this single visit.

Results: *Ex-vivo*, we have shown in this study that patients with active lesions of BON have more acidic saliva as compared to those without BON or in those not taking a BP.

Conclusion: These findings suggest that acidic salivary pH is directly associated with BON, may play a role in the initiation and prolongation of oral BON and may eventually support the potential role of using salivary buffers to offset acidic saliva as an adjunct therapeutic treatment for BON, specifically to speed wound healing.

Keywords: Zoledronic acid; Acid; Bisphosphonate

The Role of pH in Patients Receiving Long Term Bisphosphonate Therapy

By

Munawer AlHouli

Thesis submitted to the Faculty of the Graduate School of the

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Dedication

To my love:

My wife who prepared the suitable climate for me in order completed my study and to break through many odds. She was and she is everything in my life.

My mother who tolerates my alienation for years shedding tears, it’s enough I’m coming back.

Acknowledgments

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Introduction:

Every year, an estimated 20 million bisphosphonate (BP) prescriptions are written in the U.S. alone. [17, 23] BPs are synthetic analogues of the naturally occurring pyrophosphate which are capable of binding calcium and phosphate ions. [13, 36] One of the more recently reported serious adverse effects of BP treatment is BP-associated osteonecrosis of the jaw (BON), a condition first recognized in 2003. [38] Currently, the reported incidence ranges from 1.3% up to 19%, a number which has risen over time. [4-7, 9, 26, 37, 49, 52] To date, neither the pathophysiologic mechanisms leading to the induction of BON, nor treatment options for BON have been elucidated.

We recognize that most studies have focused on the effects of BPs on bone alone and the induction of BON, as BPs are preferentially deposited in areas of bone formation, specifically the mandible. [15, 21] Initially, these studies focused on BPs ability to inhibit angiogenesis, [8, 22, 40, 41] their direct apoptotic effects on osteoclasts, [34, 44] disturbance of intracellular osteoclast vesicular trafficking, [2, 34, 35, 44] and alterations of the differentiation of bone marrow osteoclast precursors. [19, 34] Overall, these mechanisms would allow BPs to stimulate the persistent suppression of bone turnover and over time create an accumulation and persistence of bony micro-damage and infection through the

inhibition of specific factors that promote bone formation. [3, 25, 33] Prior to our knowledge of BON, BPs mechanism of action at the cellular level

suggested that the microenvironment surrounding active osteoclasts was highly acidic inducing the release of the BP from the bone surface, creating high local BP concentrations and local acidity. [30, 35, 53]

Certainly, a major clinical consequence of BON is delayed wound healing, hypothesized to be either secondary to the toxic effects of BP itself, effects of BP on vascularity and bone remolding, immune regulation, microbial colonization and/or an acidic microenvironment. [1, 31, 35, 43] It is unfortunate that the term osteonecrosis endured with this condition intimating an issue involving bone alone and ignoring the cross talk between bone, drug and oral mucosal soft tissue. This condition may be better described as chemonecrosis, implicating a role of microbial aspects and soft tissue effects, in addition to the effects on bone, especially in wound healing. [18]

Unrelated to our more recent knowledge of BON, it was theorized that the acidity present is localized areas of the oral cavity could secondarily cause the release of BP from bone. [30, 35] Certainly, osteoclast activity and the presence of infection could increase the acidity present in localized inflamed oral sites, including BON lesions. [29] We believe this process contributes to a possible mechanism of delayed wound healing. In Drs Meiller and Scheper's laboratories, they have previously shown that free BP does indeed cause apoptosis and inhibit cell proliferation at micromolar (μM) concentrations. [43] Additionally, they have shown that when bound to bone, BP is non-toxic, but becomes so when released. [42] Hence, at least two processes are likely involved with the initiation

of BON, including both BP release in acidic microenvironment and the acidity itself.

Clinically, BON is observed as a mucosal dehiscence leading to the formation of a superficial mucosal ulcer, which typically progresses and results in detectable bony exposure. The ulcerated area continues to extend with time, exposing bone necrosis and sequestration. Once a BON lesion fully develops, the clinical issues revolve around the microbial populations and soft tissue wound healing. This has led to the current American Association of Oral and Maxillofacial Surgery (AAOMS) guidelines for managing BON which includes the use of chlorhexidine and antibiotics for the treatment of symptomatic BON and surgery only when lesion progression is present. [37] The clinical experience with over a hundred BON patients in the Oral medicine Program, shows that those lesions which heal do so secondary to epithelial migration under the exposed bone until that necrotic bone (determined histologically) sequesters leaving an immature, healing soft tissue bed.

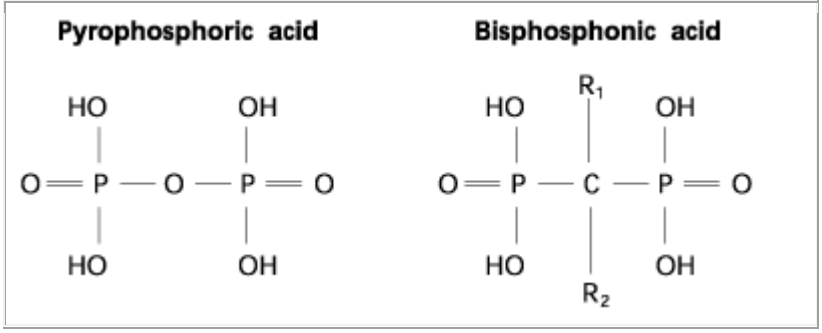
These unresolved issues of BON have led to develop a multilateral soft tissue model of BP accumulation in bone and the effects of an acidic environment and free BP on oral soft tissue mucosa. A multi-factorial cascade must exist, including a combination of the long term profound effects on bone, development of a microbial smear layer and oral mucosal soft tissue breakdown, all delaying wound healing. The previous findings in the Meiller Scheper laboratories have led us to the focus of this Masters project assessing the salivary pH in a variety of patients with and without BON to determine if any

relationship may exist and may be worth exploring pH neutralization as an adjunct to other BON therapies.

Background

Bisphosphonates are compounds with a chemical structure similar to that of inorganic pyrophosphate, and endues regulate mineralization. Inorganic pyrophosphate is comprised of two phosphate groups linked by phosphoanhydride bonds (aP-O-P structure), bisphosphonates are comprised of two phosphate groups linked by phosphoether bonds to a central (germinal) carbon atom (aP-C-P structure). The (aP-O-P) bonds are not stable. The(aP-C-P) is highly resistant to hydrolysis under acidic conditions or by pyrophosphatases, two additional covalent bonds to the germinal carbon atom of bisphosphonates can be formed with carbon, oxygen, halogen, sulfur, or nitrogen atoms, giving rise to an enormous range of possible structures. The two covalently bonded groups attached to the germinal carbon are usually referred to as $R^1$and R^2.

Fig. 1 Chemical structure of pyrophosphate and bisphosphonates [88]



Bisphosphonates form a three dimensional structure capable of binding divalent metal ions such as Ca^{2+} , Mg^{2+}, and Fe^{2+} in a bidentate manner, by coordination of one oxygen from each bisphosphonate group with the divalent cation. The affinity for calcium can be increased further if one of the side chain R^{1+} is a hydroxyl (-OH) or primary amino tridentate conformation that is able to bind Ca^{2+} more effectively. [54]

Following absorption bisphosphonates, which are very hydrophilic, are rapidly cleared from the circulation, about 50% binding to the hydroxyapatite bone mineral and 50% being excreted in the urine in part by an active secretion process.

The half-life in the circulation is about one hour or less and over 90% of the bisphosphonate not retained on the bone surface is excreted within the first 24 hours.

Bisphosphonates have been shown to increase bone mineral density at the hip and lumbar spine, and additionally, to reduce the risk of fracture at bone sites. [55]

The bisphosphonate currently available includes an oral agent form that may be administered weekly or monthly (alendronate, risedrotane, and ibandronate). And the intravenous formulation that are administered quarterly or annually (ibandronate and zoledronic acid, respectively). [56]

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Intravenous Bisphosphonates:

Bisphosphonate is used in an intravenous form mainly due to its effectiveness in the treatment and management of cancer related condition as hypercalcemia of malignancy, skeletal- related events associated with bone metastases in the context of solid tumors such as breast cancer, prostate cancer and lung cancer, and management of lytic lesions in the setting of multiple myeloma.[57-68] While it hasn't been proven yet that bisphosphonates improve cancer-specific survival, they have had a significant positive effect on the quality of life for patients with advanced cancer involving the skeleton. Before 2001, pamidronate (Aredia®) was the only drug approved in the United States for treatment of metastatic bone disease. In 2002, zoledronic acid (Zometa®) was approved for this indication by the US Food and Drug Administration (FDA). [68] More recently, a once yearly infusion of zoledronate (Reclast®) and a parenteral formulation of ibandronate (Boniva®) administered every three months have been approved by the FDA for management of osteoporosis. [69]

Oral Bisphosphonates.

Oral bisphosphonates are used to treat osteoporosis as well as being frequently used to treat osteopenia. [70] They are also used for a variety of less common conditions such as Paget's disease of bone, and osteogenesis imperfecta of childhood. [71-72] however the most common indication, is osteoporosis. [73-74] Osteoporosis may arise in the context of other diseases such as inflammatory bowel disease or primary biliary cirrhosis, as the result of medications, most commonly steroids, or as a consequence of postmenopausal

aging.[75-77]

Previously, studies have identified significant differences of mineral binding affinity and FPPS enzyme inhibition between individual bisphosphonates and it may account for the differences in clinical effects between individual members of this drug class. Zoledronate has been found to have the highest affinity to bone mineral and the following rank order for mineral affinity has been established: clodronate < etidronate < risedronate < ibandronate < alendronate < pamidronate < zoledronate). FPPS enzyme inhibition also varies between individual bisphosphonates with zoledronate having the greatest inhibitory effect (rank order: etidronate = clodronate (extremely weak inhibitors) <<<< pamidronate < alendronate < ibandronate < risedronate < zoledronate). The effect of bisphosphonate binding to bone on surface charge (zeta potential) has also been studied and is thought to influence the further binding of the bisphosphonate to bone and impact on drug accumulation. The following rank order has been reported with alendronate reported to have the greatest effect on binding capacity: risedronate < clodronate < etidronate < zoledronate < ibandronate < alendronate. Zoledronate is the most clinically potent bisphosphonate with both potent biochemical actions and a high level of affinity to bone mineral.

Bisphosphonates have a high affinity for bone mineral and bind strongly to hydroxyapatite resulting in selective uptake to the target organ and high local concentration in bone, particularly at sites of active bone remodeling. Bisphosphonates act by inhibiting osteoclast cell function resulting in reduced

bone resorption. Osteoblast and osteocyte apoptosis may reduce by bisphosphonates. Non-nitrogen which contain bisphosphonates and nitrogen which contain bisphosphonates are the main bisphosphonates forms. Non-nitrogen containing bisphosphonates (etidronate, clodronate and tiludronate) act by interacting with ATP in osteoclasts forming ATP analogues that induce osteoclast apoptosis. Nitrogen-containing bisphosphonates inhibit farnesyl pyrophosphate synthase (FPPS), a key enzyme in the mevalonic acid pathway, in osteoclasts which prevents the production of proteins essential for their function and survival. Inhibition of this enzyme also leads to an accumulation of isopentenyl diphosphate (IPP) which is incorporated into an analogue of ATP that can induce osteoclast apoptosis. Nitrogen-containing bisphosphonates can be further divided into 2 groups: alkyl-amino bisphosphonates (pamidronate, alendronate, and ibandronate) and heterocyclic bisphosphonates (risedronate and zoledronate). In addition to inhibiting FPPS, the heterocyclic bisphosphonates form a more stable enzyme-bisphosphonate complex which increases the inhibitory potency of these drugs. [78]

Disadvantages:

Oral type	Intravenous type
Esophageal and Gastric irritation	I/V is for patient who has problem taking as
abdominal discomforts	bisphosphonate orally
Gastroesophageal reflex	Arthralgia
Chest pain	Myalgia and fever following

Zoledronic acid

[56]

One of the major disadvantages of bisphosphonate in dentistry is Osteonecrosis of the Jaws (ONJ). Osteonecrosis of the Jaw (ONJ) is characterized by denuding of the squamous oral mucosal in an area of the mandible or maxilla causing an area of exposed bone. The exposed bone can be associated with pain or infection, or may be asymptomatic. [79]

The American Association of Oral and Maxillofacial Surgeons staging system for bisphosphonate associated jaw osteonecrosis:

At risk No apparent necrotic bone in patients who have been treated with either oral or IV bisphosphonates

Stage 0 No clinical evidence of necrotic bone, but non-specific clinical findings and symptoms

Stage 1 Exposed/necrotic bone in asymptomatic patients without evidence of infection

Stage 2 Exposed/necrotic bone associated with infection as evidenced by pain and erythema in region of exposed bone with or without purulent discharge

Stage 3 Exposed/necrotic bone in patients with pain, infection, and one or more of the following: exposed and necrotic bone extending beyond the region of alveolar bone resulting in pathological fracture, extraoral fistula, oral antral/oral nasal communication, or osteolysis extending to the inferior border of the mandible or the sinus floor. [80]

Many studies show that the ONJ lesions are more commonly found in

mandible more than in maxilla (2:1 ration) and areas with thin mucosa covering bony prominences such as tori, bony exostoses and the mylohyoid ridge, are the common areas where ONJ lesions can be occur .[81-83] Why is bisphosphonate-induced osteonecrosis in the jaw restrictively?[78] The restriction of bisphosphonate-induced osteonecrosis to the jaw is related to the unique nature of the blood supply, structure, function and microbiology of the jaw bones . The jaw bones have a high blood supply which may result in an increased concentration of bisphosphonates in this area. Bone turnover is also thought to be high in the jaw due to forces related to chewing and the presence of teeth.

Dental procedures and dental diseases may also increase bone turnover. The jaw bones may be more vulnerable to trauma and infection as there is only a fragile barrier of a thin mucosa and the periosteum between the jaw bones and the external environment. Furthermore, teeth are only separated from bone by thin connective tissue which may allow easy access for bacteria. There may also be differences within the jaw and between the mandible and maxilla.

The risk of ONJ associated with oral bisphosphonate therapy for osteoporosis is Low, estimated between 1 in 10,000 and <1 in 100,000 patient-treatment years. Risk of ONJ in patients with cancer treated with high doses of intravenous bisphosphonates is high, in the range of 1–10 per 100 patients (depending on duration of therapy). [84]

A large number of review of cases of Osteonecrosis of the jaw (ONJ) in

patients taking bisphosphonate brought to light that the formidable majority (85%) of patients taking bisphosphonate who experienced ONJ were taking the medication as part of their malignancy treatment (hypercalcemia of malignancy or metastatic disease), and 94% of these patients were receiving intravenous pamidronate or zoledronic acid at higher doses than would be administered to a patient being treated for osteoporosis. [52]

Patients receiving IV bisphosphonates and undergoing dentoalveolar surgery are at least seven times more likely to develop BRON than patients who are not having this kind of surgery. [85, 86] Invasive dental work in period of temporary or permanent bisphosphonate treatment is not recommended by The American Dental Association (ADA). [87]

Materials and Methods:

Experimental Design: This was a single sample saliva collection and analysis acquired from patients presenting to the University of Maryland, Multi-Disciplinary BON clinic and from the Oral Medicine Program. All of the experiments in this study were designed to look at the *ex-vivo* effector mechanisms of wound healing in BON.

Acidity of Saliva and BP: Using the fully IRB approved HIPAA partial waiver for recruitment, subjects were selected based on history and physical examination in order to document previous and/or current use of bisphosphonates. Standard of care physical examination was used to determine the presence or absence of BON lesions intraorally. As part of a fully IRB approved protocol the informed consent process was used and for those willing to participate and having signed the consent document, we collected the single sample of saliva. We employed ex-vivo studies using pH measurement on ~5 ml of fresh unstimulated whole saliva collected over a 5 minute time period at this single visit.

Clinically, saliva was collected from a total of 90 adult patients including the following subgroups: 15 normal patients (no cancer, no BP therapy, no BON), 15 osteoporosis patients taking oral-BP (alendronate/ Fosamax) without BON, 5 osteoporosis patient taking oral-BP (alendronate/ Fosamax) with BON and 55 cancer patients taking IV BP (zoledronic acid/ Zometa) having multiple myeloma, breast or prostate cancer (40 with BON and 15 without BON).

Unstimulated whole saliva was obtained by 5 minute expectoration into a sterile polypropylene centrifuge tube. Subjects refrained from eating, drinking, chewing gum, etc., for at least 1 to 2 hours prior to collection. Samples were obtained by requesting subjects to swallow first, tilt their head forward, and spit all saliva into the 50-ml tube for 5 minutes without swallowing. The tubes were placed in an alcohol/ice bath following the collections.

All samples were immediately transported to the laboratory, centrifuged at 4°C, 6000 g for 20 minutes to remove the cellular debris and then the supernatant was aliquoted into 100 µl tubes and pH was measured. The pH was measured using both Micro pHydrion insta-chek 0-13 test strips (MicroEssential Laboratory, Brooklyn, NY, USA). All samples were stored at −80°C for later analysis. All samples in this study were collected under the approved IRB protocol.

Two vials of fresh ZA injectable (Zometa®, Novartis Pharmaceuticals Corp, East Hanover, NJ) in infusion solution (non-calcium containing infusion solution, 0.36% saline) were also measured for pH. Fresh ZA injectable (Zometa®, Novartis Pharmaceuticals Corp, and East Hanover, NJ) in infusion solution (non-calcium containing infusion solution, 0.36% saline) was tested at concentrations:

0.25 to 10μM up to 48 h (pre-concentration baseline plasma level is 1μM). The concentrations were selected because they are clinically relevant in patients receiving ZA, as representative of the lower limits of estimated plasma concentrations following a 15 minute infusion. [12, 45-47]

Results:

Salivary pH in patients: Clinically, saliva was collected from a total of 90 adult patients including the following subgroups: 15 normal patients (no cancer, no BP therapy, no BON), 15 osteoporosis patients taking oral-BP (alendronate/ Fosamax) without BON, 5 osteoporosis patient taking oral-BP (alendronate/ Fosamax) with BON and 55 cancer patients taking IV BP (zoledronic acid/ Zometa) having multiple myeloma, breast or prostate cancer (40 with BON and 15 without BON).

In the control groups, patients who had never taken BPs, 14 of 15 had basic saliva at measurement (one 6.5 pH; fourteen 7.0-8.0 pH) and the average is 7.3pH. [Table. 1]

Table 1: group of 15 normal patients which have no cancer, no BON, and did not receive exposure to bisphosphonate.

RES #	AGE	RACE	SEX	BP	Osteo/Ca	BON	LOC	smoking	SAL pH	Diab
76	59	3	1	-	n	0	0	.	7.4	.
77	77	2	2	-	n	0	0	.	7.5	0
78	59	1	1	-	n	0	0	.	7.3	.
79	66	3	2	-	n	0	0	.	7.3	0
80	49	1	1	-	n	0	0	.	7.4	.
81	62	1	2	-	n	0	0	.	7	0
82	39	1	2	-	n	0	0	.	7.5	.
83	71	1	2	-	n	0	0	.	7.5	0
84	55	1	2	-	n	0	0	.	7	0
85	65	1	2	-	n	0	0	.	6.5	.
86	63	2	1	-	n	0	0	.	7.5	0
87	59	1	2	-	n	0	0	.	7.5	.
88	58	1	2	-	n	0	0	.	7.5	.
89	52	1	2	-	n	0	0	.	7	0
90	66	1	2	n	n	0	0	.	8	.

Keys: Race 1= C.C 2= A.A 3= Asian Sex 1= Male 2= Female Bisphosphonate A= Fozamax Osteo/Ca b= Breast p= Prostate mm= Multiple Myeloma O= Osteoporosis N= normal BON 1= positive 0= negative LOC[location of the lesion] 1= mandible 2= maxilla 3=both Smoking 1= smoker 0= non smoker .= not collected Diab 1= positive 0= negative .= not collected

In an additional BP exposure/risk control, all 15 patients who were taking oral alendronate without BON lesions had basic saliva at measure (AV 7.3 pH range 7.1-7.6). [Table.2]

Table 2: group of 15 patients which have osteoporosis, no BON, and exposed to bisphosphonate as A.

RES #	AGE	RACE	SEX	BP	Osteo/Ca	BON	LOC	smoking	SAL pH	Diab
46	57	1	2	a	o	0	0	.	7.1	.
47	72	1	2	a	o	0	0	.	7.2	.
48	55	1	1	a	o	0	0	.	7.1	0
49	64	1	2	a	o	0	0	.	7.6	.
50	59	1	2	a	o	0	0	.	7.3	.
51	77	1	2	a	o	0	0	.	7.5	1
52	66	1	2	a	o	0	0	.	7.1	.
53	61	1	2	a	o	0	0	.	7.2	0
54	59	1	1	a	o	0	0	.	7.1	0
55	71	1	2	a	o	0	0	.	7.4	0
56	70	1	2	a	o	0	0	.	7.1	0
57	67	2	2	a	o	0	0	.	7.3	0
58	66	1	2	a	o	0	0	.	7.1	0
59	55	1	2	a	o	0	0	.	7.3	.
60	63	1	2	a	o	0	0	.	7.2	0

Keys: Race 1= C.C 2= A.A 3= Asian Sex 1= Male 2= Female Bisphosphonate A= oral alendronate

Osteo/Ca b= Breast p= Prostate mm= Multiple Myeloma O= Osteoporosis BON 1= positive 0= negative LOC[location of the lesion] 1= mandible 2= maxilla 3=both Smoking 1= smoker 0= nonsmoker .= not collected Diab 1= positive 0= negative .= not collected

In the last BP exposure/risk control group, all had taken IV ZA and had no BON lesions. In this group 3 of 15 had acidic saliva at measurements (all 6.5 pH). The remaining 12 subjects without BON had an average 7 pH with a range 7.0-7.8[Table.3]

Table 3: group of 15 patients which have cancer, no BON, and exposed to bisphosphonate as Z.A.

RES #	AGE	RACE	SEX	BP	Osteo/Ca	BON	LOC	smoking	SAL pH	Diab
61	67	1	2	z	b	0	0	.	6.5	.
62	79	1	2	z	b	0	0	.	7.2	0
63	81	2	2	z	b	0	0	.	7	.
64	66	2	2	z	mm	0	0	.	7	0
65	59	1	2	z	mm	0	0	.	6.9	0
66	49	1	2	z	b	0	0	.	7	.
67	62	1	1	z	p	0	0	.	7	.
68	74	1	1	z	p	0	0	.	7.5	0
69	61	2	2	z	b	0	0	.	6.5	0
70	58	2	2	z	b	0	0	.	7.2	.
71	72	1	2	z	mm	0	0	.	7.4	1
72	66	1	2	z	b	0	0	.	7.2	.

73	71	1	2	z	b	0	0	.	7.8	0
74	84	1	1	z	p	0	0	.	7	0
75	63	1	2	z	mm	0	0	.	6.5	0

Keys: Race 1= C.C 2= A.A 3= Asian Sex 1= Male 2= Female Bisphosphonate Z= zoledronic acid Osteo/Ca b= Breast p= Prostate mm= Multiple Myeloma O= Osteoporosis BON 1= positive 0= negative LOC[location of the lesion] 1= mandible 2= maxilla 3=both Smoking 1= smoker 0= nonsmoker .= not collected Diab 1= positive 0= negative .= not collected

In the BP groups with BON, one patient who had taken alendronate and developed BON had a saliva pH of 6.5 and the remaining 4 subjects ranged from 6.9-7.5 pH. [Table 4]

Table 4: group of 5 patients which have osteoporosis, BON, and exposed to bisphosphonate as A.

RES #	AGE	RACE	SEX	BP	Osteo/Ca	BON	LOC	smoking	SAL pH	Diab
41	57	1	2	a	o	1	3	.	6.5	0
42	81	2	1	a	o	1	1	.	6.9	.
43	62	2	2	a	o	1	1	.	6.9	0
44	47	1	2	a	o	1	1	.	7.5	.
45	56	1	2	a	o	1	1	.	7	0

Keys: Race 1= C.C 2= A.A 3= Asian Sex 1= Male 2= Female Bisphosphonate A= oral alendronate Osteo/Ca b= Breast p= Prostate mm= Multiple Myeloma O= Osteoporosis BON 1= positive 0= negative LOC[location of the lesion] 1= mandible 2= maxilla 3=both Smoking 1= smoker 0= nonsmoker .= not collected Diab 1= positive 0= negative .= not collected

Moreover, 40 patients all had taken IV ZA and had active BON lesions. In this group 24 of 40 had acidic saliva at measurement (av. 5.9 pH with a range of 5.0-

6.5 pH), and the remaining 16 subjects had av. 7.4 pH with a range of 7.0-8.5 pH. [Total AV =6.3pH] [Table 5]

Table 5: group of 40 patients which have cancer, BON, and exposed to bisphosphonate as Z.A.

RES #	AGE	RACE	SEX	BP	Osteo/Ca	BON	LOC	smoking	SAL pH	Diab
1	72	1	2	z	b	1	1	0	5	.
2	58	2	1	z	p	1	1	1	5.5	0
3	75	2	1	z	mm	1	1	.	6.5	1
4	82	2	1	z	mm	1	1	0	5	0
5	81	2	2	z	b	1	1	1	6.5	.
6	64	1	1	z	p	1	1	0	7	.
7	67	1	2	z	mm	1	3	.	6.5	.
8	53	2	2	z	b	1	1	1	7	1
9	63	2	1	z	mm	1	1	1	7.5	0
10	63	1	2	z	b	1	1	1	6	.
11	43	2	2	z	b	1	1	0	6.5	1
12	67	2	2	z	b	1	2	.	8	.
13	62	2	1	z	p	1	2	1	7	1
14	67	2	2	z	b	1	1	0	5	1

15	79	2	2	z	b	1	1	0	6.5	.
16	57	1	2	z	mm	1	1	0	7.5	0
17	56	1	2	z	b	1	1	1	7.5	.
18	87	2	2	z	b	1	3	0	6	0
19	81	1	2	z	b	1	1	0	6	.

[Table 5Continued]

20	87	2	2	z	mm	1	1	0	7	1
21	57	2	1	z	mm	1	1	.	7	0
22	67	2	2	z	b	1	1	0	5	.
23	70	1	1	z	mm	1	1	1	5	0
24	71	2	2	z	b	1	1	.	6	.
25	52	2	2	z	b	1	1	1	7	0
26	82	1	1	z	mm	1	2	1	6.5	.
27	47	1	2	z	mm	1	1	0	8.5	.
28	86	2	1	z	mm	1	2	0	5	.
29	71	2	2	z	b	1	1	.	7	.
30	56	1	1	z	mm	1	1	1	6	1
31	76	2	1	z	mm	1	3	1	7.5	0
32	61	1	2	z	b	1	3	1	5	0
33	57	2	2	z	b	1	1	.	5.5	1
34	85	1	2	z	mm	1	1	0	5	0
35	84	1	1	z	mm	1	1	1	5	.

36	81	1	2	z	b	1	1	0	7	1
37	51	2	2	z	b	1	1	.	7.5	1
38	42	1	2	z	mm	1	1	0	5	.
39	64	1	2	z	mm	1	2	1	6.5	0
40	62	1	2	z	b	1	1	.	6.5	.

Keys: Race 1= C.C 2= A.A 3= Asian Sex 1= Male 2= Female Bisphosphonate Z= zoledronic acid Osteo/Ca b= Breast p= Prostate mm= Multiple Myeloma O= Osteoporosis BON 1= positive 0= negative LOC[location of the lesion] 1= mandible 2= maxilla 3=both Smoking 1= smoker 0= nonsmoker .= not collected Diab 1= positive 0= negative .= not collected

It is known that ZA itself is acidic with a pH of between 6 and 7. (Labeling, Novartis Pharmaceuticals Corp., East Hanover, NJ, USA) Looking at 2 consecutive fresh discard ZA injectable drug samples in non-calcium containing infusion solution showed the 2 drug samples themselves both had a pH 6.0 respectively. [Table. 6]

Table 6: group of 2 samples of bisphosphonate, zolodronic acid in infusion liquid ZI.

RES #	AGE	RACE	SEX	BP	Osteo/Ca	BON	LOC	smoking	SAL pH	Diab
91				ZI					6	
92				ZI					6	

Keys: Race 1= C.C 2= A.A 3= Asian Sex 1= Male 2= Female Bisphosphonate= ZI Osteo/Ca b= Breast p= Prostate mm= Multiple Myeloma O= Osteoporosis N= normal BON 1= positive 0= negative LOC[location of the lesion] 1= mandible 2= maxilla 3=both Smoking 1= smoker 0= nonsmoker .= not collected Diab 1= positive 0= negative .= not collected

Each group had deferent patient number and each patient has his own pH level data.

All pH data in each group were collected together and the results were divided on the number of patients in the same group. These calculations were done to get pH level average in each group which helps us to illustrate and compare their data. In Table 7 all groups were listed with their pH average .

[Table 7] pH average for all groups.

Group number	Medical condition	Bisphosphonate type	BON	pH Average
1 [40]	Cancer	Intravenous	+	6.3
2[5]	Osteoporosis	Oral	+	6.9
3[15]	Osteoporosis	Oral	_	7.24
4[15]	Cancer	Intravenous	_	7
5[15]	Normal	_	_	7.3

6[2]	Normal	Intravenous Z.A	_	6

Keys += BON is positive -= BON is negative

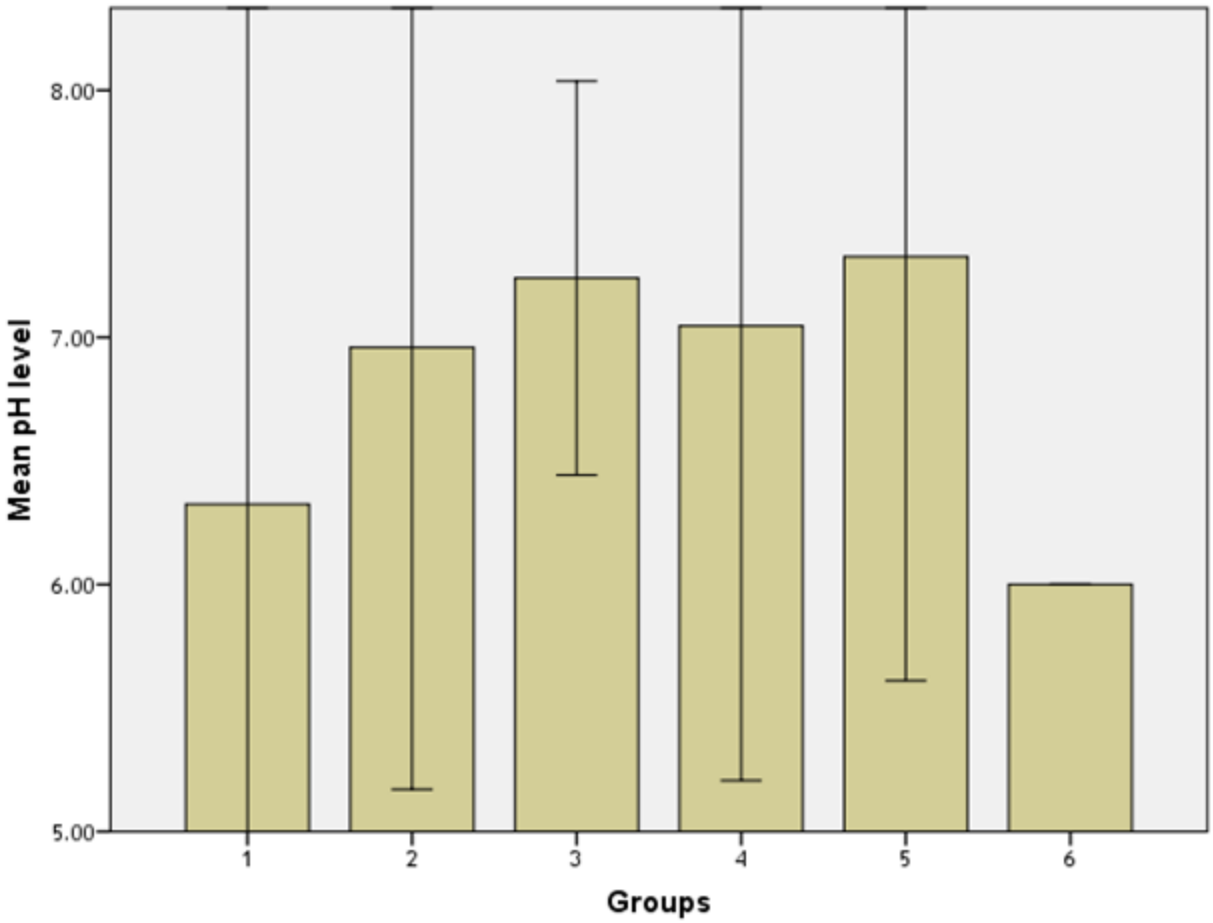
Culiom1= Group1 (patients with BON and using IV BP). Culom2=Group2 (patients with BON using oral BP)

Culom3=Group3 (patients without BON and using oral BP). Culom4=Group4 (patients without BON using IV BP)

Culom5=Group5 (patients have no BON and did not treated by BP [normal patients]) Culom6= samples of ZI drugs.

After we had the pH average for each group, it's easy now to make the graphic [Fig.2]

[Fig.2] The pH average for each group

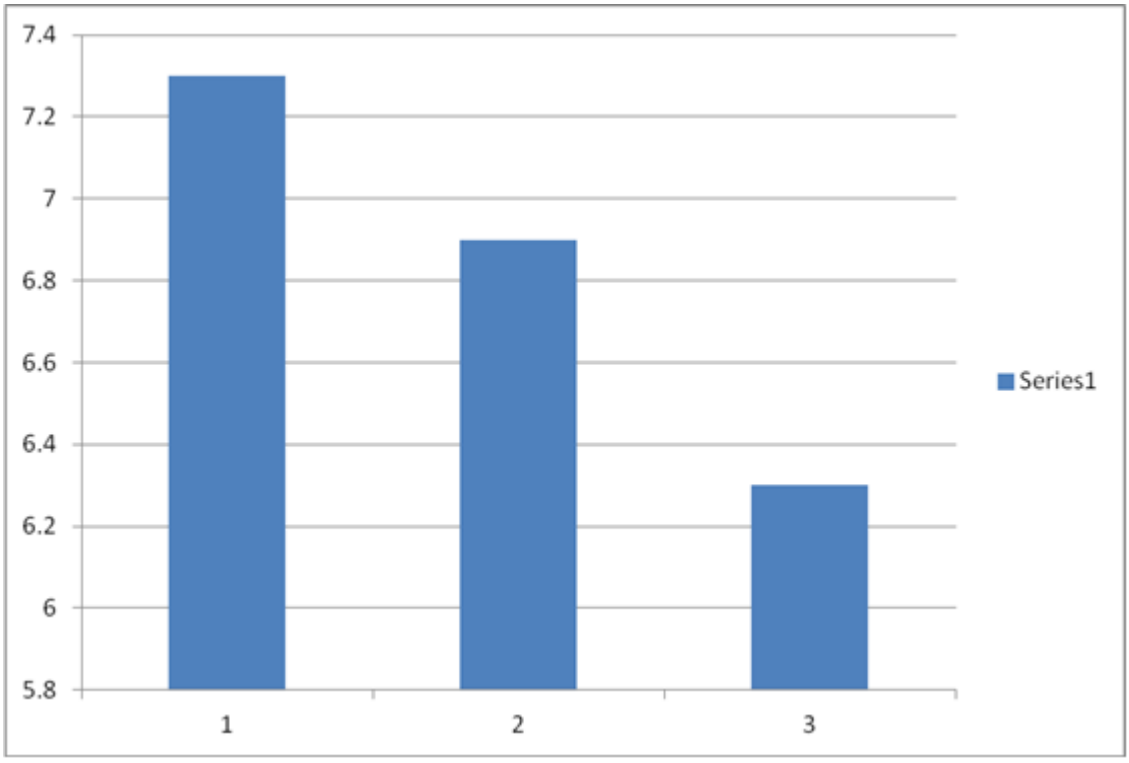


Culom1= Group1(patients with BON and using IV BP). Culom2=Group2(patients with BON using oral BP) Culom3=Group3(patients without BON and using oral BP). Culom4=Group4(patients without BON using IV BP) Culom5=Group5(patients have no BON and did not treated by BP[normal paitents]) Culom6= samples of ZI drugs.

Comparing the pH averages can give us more information about the role of pH in BON. The pH average in normal patients is 7.3 pH, the pH average in cancer patients with BON having intravenous bisphosphonate is 6.3 pH and the pH average in osteoporosis patients with BON having oral bisphosphonate is 6.9pH. That mean is the normal patients have normal, non-acidic oral environment, but

other two groups have lower pH level which mean the oral environment is acidic as it shown in the next table. [Fig. 3]

[Fig. 3] Comparing the pH averages of patients have no BON and did not treated by BP [normal patients], patients with BON using oral BP and patients with BON and using IV BP).



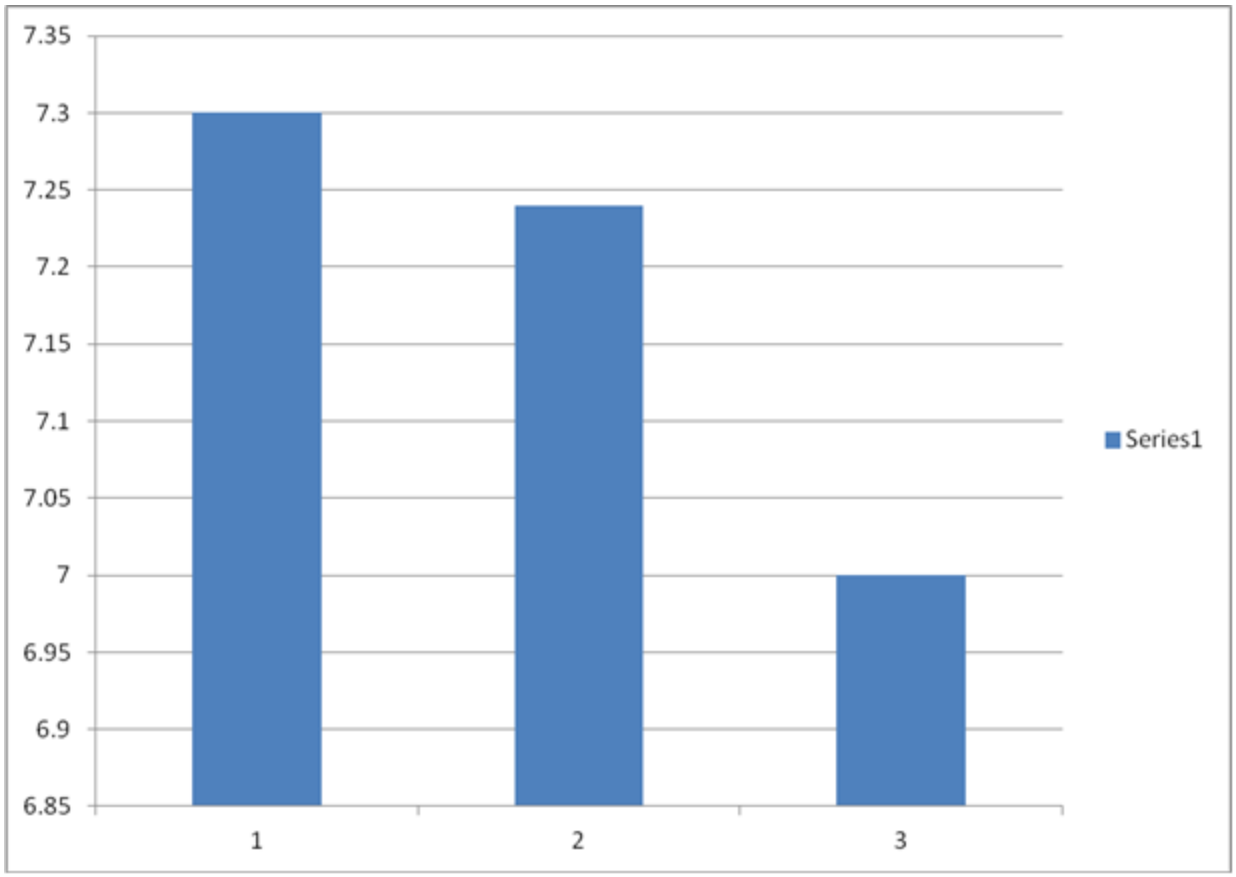
Culom1=Group5 (patients have no BON and did not treated by BP [normal patients])

Culom2=Group2 (patients with BON using oral BP) Culom3= Group1 (patients with BON and using IV BP).

Patients with intravenous bisphosphonate are in more risk because the average pH is 6.3 comparing to patients with oral bisphosphonate (average pH is 6.9) shows that intravenous bisphosphonate patients are in higher risk than the oral bisphosphonate patients. Also, the pH average in patients without BON but they

were treated by intravenous bisphosphonate is 7 pH, while the patients without BOJ but they were treated with oral bisphosphonate pH average is 7.24. [Fig.4]

[Fig.4] Comparing the pH averages of patients have no BON and did not treated by BP [normal patients], patients without BON and using oral BP and patients without BON using IV BP

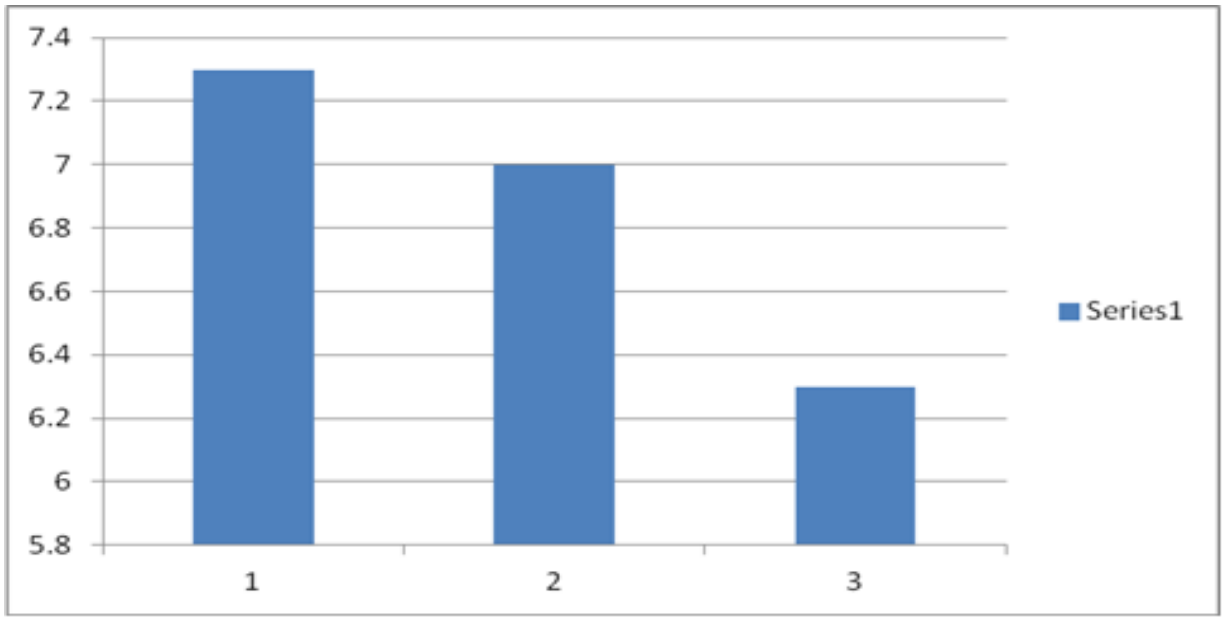


Culom1=Group5 (patients have no BON and did not treated by BP [normal patients])

Culom2=Group3 (patients without BON and using oral BP). Culom3=Group4 (patients without BON using IV BP)

That graph can give us information that conform patients receiving intravenous treatment are more likely in danger to have BON and have acidic oral environment. That explains the reason of the low pH (6) in the last two samples. A couple comparisons should be done and in this one we will compare two group of patients receiving the same type of BP. Cancer Patients without BON having I.V BP average pH level is =7 and cancer patients with BON having I.V BP average pH level is= 6.3. [Fig.5]

[Fig.5] Comparing the pH averages of patients have no BON and did not treated by BP [normal patients], patients without BON using IV BP and patients with BON and using IV BP.



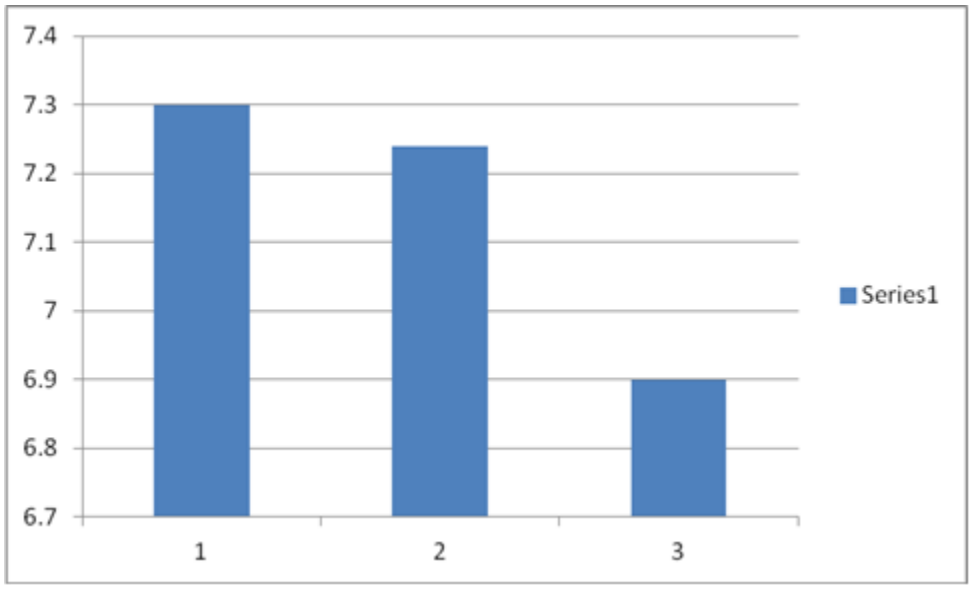
Culom1=Group5 (patients have no BON and did not treated by BP [normal patients])

Culom2=Group4 (patients without BON using IV BP) Culom3= Group1 (patients with BON and using IV BP).

The second group of patients have lower (acidic environment) pH level.

Osteoporosis patients without BON and having oral BP average pH level is= 7.24 and osteoporosis patients with BON having oral BP average pH level is= 6.9. [Fig.6]

[Fig. 6] Comparing the pH averages of patients have no BON and did not treated by BP [normal patients], patients without BON and using oral BP and patients with BON using oral BP.



Culom1=Group5 (patients have no BON and did not treated by BP [normal patients])

Culom2=Group3 (patients without BON and using oral BP). Culom3=Group2 (patients with BON using oral BP)

All of these graphics and information from them confirms the role of pH level in BON theory.

Discussion:

It is well known that ZA, of the entire BPs, has the greatest affinity for bone. This occurs through the inhibition of farnesyl pyrophosphate synthase leading to the accumulation of BP in areas of highest bone turnover, namely the mandible where most BON lesions occur. [39] In these areas of high bone turnover, an increase in surface BP along with decreased diffusion into bone would occur. [39] Additionally, it is known that ZA does not completely inhibit osteoclasts. Chemistry dictates BPs bind to hydroxyapeptite and when challenged, the initial loss of a pyrophosphate would release free BP. [39] [24] This finding was also found with infectious agents including several residing in the oral cavity including: pseudomonas, klebsiella, bacillus, agrobacterium, fungi and fusobacterium. [39] [24] Hence, with active bone resorption and/or remolding due to the remaining active osteoclasts, a local acidic environment ensues leading to the release of free BP and uptake within the local bone cells and soft tissue cells.

In the literature an association between osteonecrosis of the jaw and bisphosphonate drugs has been found but the defined pathophysiologic mechanism behind this association and treatment regimen still unknown. Multiple factors can play important role in slow down the wound healing process in BON patients such as pH, microbial pathogens, saliva composition and soft tissue. Our study is designed to evaluate one of the possible factors (pH) that might associate with BON and delayed healing process.

In this study, our initial clinical observation showed that saliva collected from patients with active BON had significantly more acidic saliva while those never having used BPs, had a pH almost coincided with those on IV or oral BPS without BON.

We found that osteoporosis patients taking PB orally with BON (group 4) and cancer patients treated with intravenous BP with BON (group 5) have pH average =6.9 and pH average=6.3, respectively , compared with normal (group1) pH average = 7.3 (no BON and not taking BP). Patients with BON have more acidic saliva than normal group not taking BP.

On other hand, osteoporosis patients taking PB orally without BON (group 2) and cancer patients treated with intravenous BP without BON (group 3) have pH average =7.24 and pH average=7, respectively, compared to group 1, 4 and 5. So, patients taking BP without active BON have almost the same pH.

These findings have lead us to consider an overall multifactorial nature of BON; being initiated in bone, and carried forward to soft tissue, and infectious components, with the tripartite capable of an acidic environment and free BP release. This is possible considering chemical desorption, osteoclastic resorption, infection components and free BP.

These findings can lead us to use a basic buffer to reverse the acidic media and neutralize the saliva in the oral microenviroment which caused by PB

release in patients with active BON and taking PB may provide successful adjunctive care for these patients.

The only current therapeutics for BON presented in the literature have been pentoxyfylline with vitamin E and clodronate (proposed for osteoradionecrosis) or teriparatide in case reports for BON patients. [10, 11, 14, 16, 23, 28] However, both of these treatment options carry significant side effects and may be contraindicated in patients with cancer, the main population of patients with BON. Both of these treatment options coincide with our hypothesis that the effects on bone correlates with an in-balance of soft tissue synthesis and its degradation.

Conclusions:

Currently, only antimicrobial therapeutics or preventive strategies aimed at avoiding invasive oral interventions such as dental surgery and subsequent infection are the standard of care for patients with BON. Additionally, it is recommended that until healing from an invasive dental surgical treatment takes place, temporary discontinuation of BP therapy may be considered (up to 3 months). [20, 50, 51] However, the founded results in this study give us new knowledge that to keep patients pH level in their mouth as high as they can to prevent BON from occurring by drinking high pH liquids such as milk and water and to keep alcohol and low pH liquids especially patients who are receiving I.V

BP drugs.

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