MEDWIN PUBLISHERS
Committed to create value for Researchers

Nursing & Healthcare International Journal
ISSN: 2575-9981

Considerations Regarding Zinc Supplements in Micronutrient Deficient Nursing Home Patients

Kaminski MV[1]* and Mendoza JL[2]

[1]American Colleges of Surgeons and Nutrition and Holistic Wound Healing Service, USA

[2]Florida State University, USA

***Corresponding author:** Mitchell V Kaminski, American Colleges of Surgeons and Nutrition and 8975 W. Golf Rd. Apt 615 Niles, Il. 60714, USA, Tel: (847) 513-2106; Email: mvkaminski@comcast.net

Research Article

Volume 2 Issue 6

Received Date: November 19, 2018

Published Date: December 20, 2018

DOI: 10.23880/nhij-16000167

Abstract

To give or not give routine zinc supplements to patients being treated for pressure ulcers is a question fraught with controversy. The fear of causing a zinc toxicity, including anemia secondary to competition with copper and iron for absorption from the gut, is usually cited as the reason to withhold zinc supplements. There is no literature that reports changes in hematocrit and hemoglobin and serum zinc, ceruloplasmin and transferrin levels during zinc supplementation. Giving a micronutrient supplement with zinc was part of the standard treatment protocol for pressure wounds. The work reported here was a retrospective analysis and shows that even giving 100 mg elemental zinc daily for months, resulted in no change in any of these indices.

To avoid giving zinc to patients who do not need it, a zinc taste test was done. The zinc taste is a functional test. A zinc solution is sprayed into the mouth of the subject. Zinc has a vile flavor to a healthy subject without a deficiency. A patient with a zinc deficiency cannot taste zinc.

Zinc is the critical component of numerous protein mediated metabolic processes involved in structure, regulation and catalytic activity throughout the body. Further, matrix metalloproteinases (MMPs) are involved in every phase of healing and zinc is an essential component of MMP's. Supplementation in a micronutrient malnourished patient with a pressure wound is therefore very important.

How to monitor for toxicity is presented. Once again, the zinc taste is useful in that a patient without a zinc deficiency will complain of the terrible taste and gastrointestinal upset. The patient will refuse to take another dose.

Keywords: Metalloproteinases; Ceruloplasmin; Zinc; Iron and anemia

Abbreviations: ECM: Extra Cellular Matrix; DRI: Daily Reference Intake; TIMP'S: Tissue Inhibitor Matrix Metalloproteinases; PDGF: Platelet Derived Growth Factor; EGF: Epidermal Growth Factor; VEGF: Vascular Endothelial Growth Factor.

Introduction

Background

There are two considerations to be addressed regarding zinc supplements in patients treated for pressure wounds. First, the critical role zinc plays in health and in every phase of healing [1-3]. Second, the fear of zinc toxicity [4].

Zinc in Health and Healing

The role of zinc in health includes healing pressure ulcers but beyond that it has multiple roles. Zinc is predominantly an intracellular trace metal active throughout the body. Total body content is 1.4 - 2.5 grams [5]. Unlike other trace minerals, zinc is not stored. The zinc equilibrium is stabilized by regulating absorption vs gut elimination [6]. Zinc is critical in three areas: 1) structure, 2) regulation and 3) catalytic activity of proteins. In the liver there over 300 zinc dependent enzyme reactions [7]. In the skin, zinc plays its critical role in wound healing as part of 23 zinc dependent matrix metallo-proteinases (MMP's) [8]. MMPs govern the complex activities involved in healing from degradation of devitalized and damaged tissue to final epithelialization [9,10].

Zinc's role in the structure of proteins relates to a finger like molecular configuration that stabilize a number of them [11]. In addition, zinc is a powerful antioxidant. A zinc deficiency increases membrane susceptibility to oxidative damage [12].

Zinc's regulatory role relates to gene expression. It binds to DNA and influences transcription for specific genes. In this capacity it is involved in growth and development as well as cell signaling and apoptosis [13].

The focus of this paper is zinc's catalytic role in wound healing. The extra cellular matrix (ECM) in skin, subcutaneous tissue and between muscle groups is made of the basement membrane and fibers immersed in proteoglycans, non-proteoglycan polysaccharide and several fibers (collagen, elastin, fibronectin, laminin) [14]. If damaged, there is an MMP to enzymatically digest each. The primary sources of MMPs are macrophages [15]. If there is a substantial amount of necrotic debris and bacterial contamination, a biofilm will form. In response, higher levels of MMPs, particularly MMP-9 are recruited into the wound [16]. In this study the wound care protocol emphasized bed side mechanical debridement and biofilm curettage to reduce the devitalized debris and biofilm. The wound was dressed with a carbohydrate and collagen material and covered with and impervious product to keep the wound moist.

Zinc Toxicity

In patients being treated for pressure wounds the issue of zinc toxicity suffers confusion from inference not direct measurement. Published recommended doses are for healthy subjects who do not have a zinc deficiency. The reports published for severe zinc toxicity were in individuals experiencing exposure to chronic industrial contamination or neurotic behavior [17].

Toxicity is rare among individuals taking over the counter supplements. This is because early on, with self-administered overdose causes gastric distress and nausea. Further, to the individual who does not have a zinc deficiency the flavor of zinc is obnoxious. To someone who needs zinc, oral zinc is flavorless. In the nursing home a patient will refuse zinc for the same reasons.

Long term consumption of excessive zinc can produce a copper deficiency and subsequent failure of transferrin to carry iron to hemoglobin. A microcytic hypochromic anemia result [18,19].

When properly monitored, as described here, excess ingestion is avoided. Awareness and caution is the key. But, given the currently recognized and expanding appreciation of zinc in health, to prematurely discontinue or not give a zinc supplement to someone who has micronutrient deficiencies should not be an option.

Materials and Methods

139 patients were studied following referral to the wound care service for pressure wounds. A physical exam was performed documenting the location and stage of wounds. In addition, an exam for the oral and cutaneous signs of micronutrient deficiencies was done. Wasting of the dorsum of the hand, capillary fragility (purpura) and skin tears are due to collagen deficit which is due to vitamin C deficiency The hands, forearms/lower extremities were searched for purpura and skin tears. Zinc early deficiency was diagnosed by a bed side zinc taste test. Two puffs of Zicam (Scottsdale Az) were sprayed into the patients' mouth. A positive test (zinc deficiency) was concluded if the patient could not taste the vile flavor of zinc [17,20,21]. Findings were recorded on a standardized physical exam nutrition/wound data collection sheet.

The next morning and monthly thereafter, the phlebotomy technician drew blood at 4:00 AM.

It was placed on ice and kept chilled until it was assayed later that day.

Because all patients had objective sings of micronutrient deficiencies, they were started BID on a specifically designed profile of micronutrients which included 220mg of zinc sulfate (50 mg each x 2 = 100mg elemental zinc/d).

Results

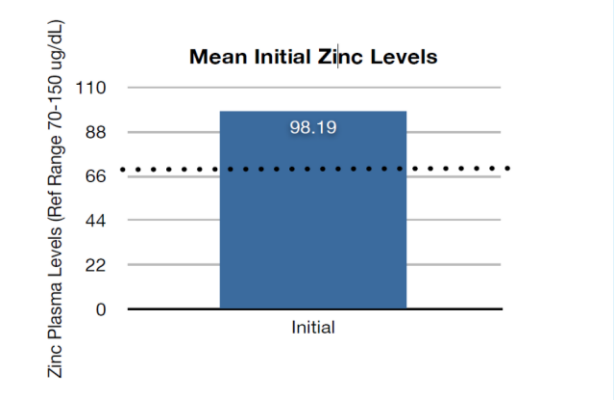

Figure 1: The mean serum zinc at the start of 220mg zinc sulfate BID dose was within the range of normal at 98.19ug/dL. (normal range 70 to 150 ug/dL). Supplements were continued to evaluate the effect of this zinc sulfate dose on change in serum level and hematopoiesis (n = 112).

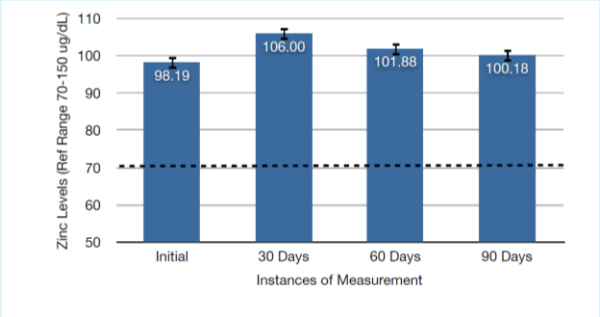

Figure 2: Over a three month period the mean serum zinc level increased. In three patients, the monthly serum value reached 100ug/dL. At that point, the dose was decreased to 220mg zinc sulfate per day. (Base line n = 117; 30 days n = 40; 60 days n = 28; 90 days, n = 14).

Figure 3: Ceruloplasmin was monitored to test the effect of zinc supplementation on copper. There was a slight mean decrease over 4 months. Ceruloplasmin levels however remained within the normal range. This was deemed expected for micronutrient malnourished patients.

(The n's were the same as above.)

Figure 4: Over a four-month period of zinc sulfate supplementation, the serum transferrin did not change. (The n's were the same as above.)

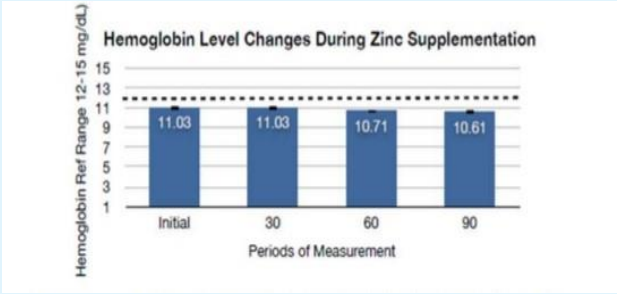

Figure 5: Giving 220 mg BID over 90 days did not affect the hemoglobin. The zinc was reduced to 220 mg/d when the serum level reached 100 ug/dL which occurred on 3 occasions (2.6%). Prolonged zinc supplementation at the doses used in this study did not affect the hemoglobin. (The n's are the same as in the above figures.)

Discussion

Zinc Requirements and Toxicity

All patients with pressure wounds have micronutrient deficiencies at the time of consult including hypovitaminosis C (Scurvy) and D. The mean serum zinc at the start of zinc supplementation was 98.19ug/dL which is low normal with the normal range given as 70 uL – 150 uL. The dose used was 100mg elemental zinc (as zinc sulfate 220 mg BID) which is 60mg higher than the 40mg upper limit recommended by the U.S. Food and Nutrition Board for healthy adults [22]. This was a deliberate decision based on the diagnosis of micronutrient malnutrition per positive physical diagnosis signs of micronutrient deficiencies on physical exam and a positive Zinc Taste Test [23]. Further, total body zinc is not stored but regulated by changes in gut absorption and urine excretion [24]. These populations are frequently on zinc wasting diuretics or suffer infections which is associated with a zincuria [25].

The RDI, the NPUAP and others do not mention these confounding variables when making recommendations for healthy adults as opposed to a nursing home population with pressure wounds. Nevertheless, base line and monthly serum zinc testing was done, and the patients were carefully mentored for intestinal upset and refusal to take the supplement which is due to zinc toxicity.

The change in mean serum zinc over time was not impressive. It neither rose nor fell. It began to trend downward at the 60- and 90-day blood draws. On the other hand, perhaps these data are significant. Where some have argued oral zinc should be discontinued after 6 weeks for fear of an overload and symptomatic toxicity, these findings suggest that recommendation be reviewed as these findings seem contrary to several other investigators as well. It cannot be over emphasized that monitoring is a must.

Giving 150mg of elemental zinc per day is common in the treatment of Wilson's disease and are well tolerated [26]. However, when given to nursing home patients' gastrointestinal disturbances were noted. These studies help narrow speculation regarding dosing range in that 150mg/d commonly causes symptoms and is 50% higher than the 100mg dose used in this paper which did not cause symptoms. Thus, 100mg per day can tentatively be regarded as a safe upper limit in micronutrient malnourished patients with pressure wounds. There is literature however that does report nausea and vomiting in a similar group of patients observed in our population at which time the author recommends the dose should be reduced to 50mg/day and not discontinued [27].

The NPUAP nutrition white paper takes a conservative view recommending that if there are clinical signs of zinc deficiency the patient can be supplemented with 40mg of elemental zinc per day which is the Daily Reference Intake (DRI) upper limit…for healthy humans. The NPUAP goes on to state that the supplement should be stopped once the deficiency is corrected. Unfortunately, a method to determine that a zinc deficiency exists or is corrected was not given. The reason given to stop zinc supplementation was to prevent "high" doses of zinc from adversely affecting copper status possibly resulting in anemia because the two minerals compete for absorption sites in the gut as well as on its carrier protein, albumin [28]. The data presented here indicates 100mg of elemental zinc given to pressure wound patients is well tolerated and may be the difference in the rate of healing with epithelialization seen in this group when anecdotally compared with services that do not supplement. That said, zinc does compete with copper for absorption and therefore a ceruloplasmin (along with transferrin and hemoglobin) should be followed monthly basis monitoring for a copper deficiency. It has been reported that some of the signs and symptoms of zinc toxicity may partly be those of an associated copper deficiency [29].

No single macronutrient or micronutrient is the culprit in delayed healing. As an example, the rapid evolution to granulation tissue observed here was more likely due to supplementing with 1gm of vitamin C/d. In other work from this group, it was noted that all patients with pressure wounds had Scurvy at the time of initial consult. Additionally, a pressure wound cannot heal unless there is pressure relief and mechanical removal of necrotic debris and biofilm on a regular basis. Wound healing is an integrated complex process. At the same time, it can be considered a chain. It is only as strong as its weakest link.

Zinc is Required for MMP's

Matrix metalloproteinases are zinc-dependent endopeptidases. They are one of a large family of proteases known as the metzincin superfamily [30]. Every protein that makes up the extracellular matrix, including the basement membrane can be digested by a specific MMP. There are 23 known MMPs [31]. They are either secreted, mostly by macrophages during the early phases of the healing process or are membrane bound. Both are activated by a common "cysteine switch" [32]. Following digestion, the protein fragments are then chaotized and

carried away. When the task is completed, there are 4 families of tissue inhibitor matrix metalloproteinases (TIMP'S) [33]. TIMP-1, TIMP-2, TIMP-3 AND TIMP-4 that neutralize the enzymatic activity [34].

Initially MMP's were named by the protein they digested but it soon became clear that a single MMP also had cross reactivity with other proteins. For example, of the 20 MMPs that can be identified in a pressure wound, many have collagenase activity [35].

A protocol that does not mechanically remove debris results in an accumulating bioburden. By definition the bioburden refers to the number of bacteria on surface that has not been sterilized [36]. Because there is no term for the complex mix of bacteria the molecules, proteins, inflammatory reactants and necrotic debris found on the surface of an indolent wound the author often refers to the noxious mix as the wound's bioburden. Simply, it is everything that should not be there to heal. It is everything that is associated with the wound being stuck in the inflammatory phase, principally all non-viable tissue debris and biofilm plus the acute inflammatory cells and secretions aimed at recovering homeostasis. It creates an endless loop until it is interrupted [37].

The biofilm is produced by a colony of bacteria covering the wound surface. The biofilm creates a gelatinous or slimy coat that protects the bacteria from antibiotics applied topically or given systemically [38]. This is why if there is no zone of erythema involving the bordering skin indicating cellulitis, wounds should not be cultured [39]. All wounds are contaminated. The bacteria "swimming in the pool" above the biofilm are called planktonic [37]. To give antibiotics for a positive culture dose nothing except encourage antibiotic resistance.

The biofilm bacteria elaborate chemokines which attract polymophonicseocytes [40]. These activated neutrophils then produce pro-inflammatory cytokines (TNFalpha, IL1, IL6, IL8, INF beta) [40-42]. Pro-inflammatory cytokines prevent migration of cells required for healing to the affected area (fibroblasts, endothelial cells and keratinocytes. Further, if *staphylococloccus aureus* is one of the bacteria in the biofilm, it inhibits macrophage phagocytosis [43].

Biofilm is adherent to any surface and requires mechanical curettage to remove it. If left in place, it encourages an indolent wound with characteristic elevated inflammatory markers, diminished growth factor activity, reduced cell numbers in the wound and elevated MMP9. These markers, including the elevation of MMP9, are the result of a "dirty wound" and not the cause of the “dirty wound". Nevertheless, in the author's opinion, it, along with untreated micronutrient deficiencies is the major cause of delayed wound healing.

Not surprisingly then, MMP-9 activity has been to inversely correlated with wound closure [37,44,45]. If there is no necrotic debris or biofilm, there is nothing to attract polymorphonucelocytes and macrophages. Decreased macrophage activity means decreased MMP-9. If MMP-9 assay shows a low level than the indolent wound may be secondary to micronutrient malnutrition [46]. In all likely hood, advanced chronic Scurvy was not recognized and treated in these cases and was the likely reason for non-healing. A wound will not heal without collagen deposition and collagen requires vitamin C. Further, if the patient has Scurvy he has multiple micronutrient deficiencies including zinc etc., all of which must be corrected to facilitate the healing process.

The toxic soup of a wound left to its own devices, will produce a self-feeding cycle furthering tissue destruction. Here the inflammatory process results in free radical generation. Hydrogen peroxide aimed at killing bacteria can also cause tissue damage further increasing the need for MMP-9 in the wound [47].

A wound also requires growth factors to granulate and epithelialize. These include platelet derived growth factor (PDGF), epidermal growth factor (EGF), and vascular endothelial growth factor (VEGF). It has been suggested that along with free radicals, excess levels of proteases such as MMP-9, also degrades growth factors [48,49].

Beyond MMPs providing its indispensable role in wound surface cleaning, angiogenesis fills in the defect. Zinc dependent MMP-1 is required for migration of fibroblasts and construction of the rich vascular network that comprises granulation tissue [50]. The epithelization of the surface is MMP-1 dependent. Epithelial cells at the edge of a wound proliferate and migrate across with the trailing epithelial cells secreting MMP-1. The keratinocytes mimic a game of leap frog. MMP-1 loosens its attachment to the basement membrane allowing it to crawl over the top of the keratinocytes in front of it, taking a new position on the edge of the advancing epithelial sheet and so on [51,52].

Finally, zinc dependent MMPs are involved in contraction and scar and remodeling. MMP's influence cell migration within the forming scar. They promote cellular proliferation as well as apoptosis and modulate growth factors. They regulate transcription factors and of course,

zinc dependent TIMPs turn off the process when the job is completed [53,54].

Summary

Wound healing requires optimal availability of zinc to support structure, function and catalytic processes throughout the body. Zinc dependent proteinases known as MMP's and their TIMPs are critical to wound healing. Recommended monitoring must be done to avoid toxicity but with that, the fear of toxicity should not be used as an excuse to under feed zinc to a micronutrient malnourished patient.

MMP-9 is present as a response to a need to clean necrotic debris and biofilm from the wound surface. Mechanical debridement/curettage is done prn to eliminate these noxious elements. Micronutrients must be provided to patients suffering micronutrient malnutrition. Associated with that is a decrease in MMP-9, and the odor and drainage produced by the vicious cycle that consists of necrotic debris begetting chemokines calling polymorphonucleocytes to the area which produces free radicles, degrading cells and reactive enzymes allowing the formation of a biofilm, chemokines etc. which will not end if unopposed. ie., the indolent wound.

Micronutrient malnutrition can be documented at the bed side in all pressure wound patients at the time of consult. This suggests a role for prevention. That is, as soon as a physical sign of a micronutrient deficiency is seen, for example chronic scurvy with its thin dermis, purpura and skin tears, supplements designed for the micronutrient malnourished individual should be started and decrease the incidence of pressure wounds.

Conclusion

Routinely withholding zinc supplements form a patient with a pressure wound for fear of toxicity is not necessary. Using regular serum monitoring, no evidence of copper or iron issues or anemia were found while giving 220mg of zinc sulfate for as long as 90 days. There were no objective signs of zinc toxicity. Monitoring is however in order beginning with an oral zinc taste test to document a functional zinc insufficiency followed by an initial and monthly serum evaluation.

References

1. Lansdown AB, Mirastschijski U, Stubbs N, Scanlon E, Agren MS (2007) Zinc in wound healing: theoretical, experimental, and clinical aspect. Wound Repair Regen 15(1): 2-16.

2. Mirastschijski U, Martin A, Jorgensen L, Sampson B, Agren M (2013) Zinc, Copper, and Selenium Tissue Levels and Their Relation to Subcutaneous Abscess, Minor Surgery, and Wound Healing in Humans. Biological Trace Element Research 153(1-3): 76-83.

3. Agren MS (1990) Studies on Zinc on Wound Healing. Acta Derm Venerel Suppl (Stockh) 154: 1-36.

4. Lemire J, Mailloux R, Appanna VD (2008) Zinc toxicity alters mitochondrial metabolism and leads to decreased ATP production in hepatocytes. Journal of Applied Toxicology 28(2): 175-182.

5. Jackson MJ (1989) Physiology of Zinc: General Aspects. In: Mills CF, et al. (eds) Zinc In Human Biology. (Chapter 1). Springer, London.

6. Krebs NF (2000) Overview of Zinc Absorption and Excretion in the Human Gastrointestinal Tract. J Nutr 130(5): 1374S-1377S.

7. Prasad AS (2013) Discovery of Human Zinc Deficiency: Its Impact on Human Health and Disease. Adv Nut 4(2): 176-190.

8. Lobmann R, Ambrosch A, Schultz G, Waldmann K, Schiweck S, et al. (2002) Expression of matrix-metalloproteinases and their inhibitors in the wounds of diabetic and non-diabetic patients. Diabetologia 45(7): 1011-1016.

9. Armstrong DG, Jude EB (2002) The Role of Matrix Metalloproteinases in Wound Healing. Journal of the American Podiatric Medical Association 92(1): 12-18.

10. Ravanti L, Kähäri VM (2000) Matrix metalloproteinases in wound repair (review). Int J Mol Med 6(4): 391-407.

11. Vallee BL, Auld DS (1990) Zinc coordination, function, and structure of zinc enzymes and other proteins. Biochemistry 29(24): 5647-5659.

12. Finamore A, Massimi M, Devirgilis L, Mengheri E (2008) Zinc Deficiency Induces Membrane Barrier Damage and Increases Neutrophil Transmigration in Caco-2 Cells. The Journal of Nutrition 138(9): 1664-1670.

13. Cousins RJ (1998) A role of zinc in the regulation of gene expression. Proceedings of the Nutrition Society Proc Nutr Soc 57(02): 307-311.

14. Hay ED (2013) Cell Biology of Extracellular Matrix, Springer Science & Buisiness Media, New York.

15. Blom AB, Lent PLV, Libregts S, Holthuysen AE, van der Kraan PM, et al. (2007) Crucial role of macrophages in matrix metalloproteinase–mediated cartilage destruction during experimental osteoarthritis: Involvement of matrix metalloproteinase 3. Arthritis Rheum 56(1): 147-157.

16. Wysocki AB, Staiano-Coico L, Grinnell F (1993) Wound Fluid from Chronic Leg Ulcers Contains Elevated Levels of Metalloproteinases MMP-2 and MMP-9. Journal of Investigative Dermatology 101(1): 64-68.

17. Plum LM, Rink L, Haase H (2010) The Essential Toxin: Impact of Zinc on Human Health. Int J Environ Res Public Health 7(4): 1342-1365.

18. Patterson WP, Winkelmann M, Perry MC (1985) Zinc-Induced Copper Deficiency: Mega mineral Sideroblastic Anemia. Ann Intern Med 103(3): 385-386.

19. Gyorffy E, Hung C (1992) Copper Deficiency and Microcytic Anemia Resulting from Prolonged Ingestion of Over--the--Counter Zinc. Am J Gastroeneterol 87(8): 1054-1055.

20. Aliani M, Udenigwe CC, Girgih AT, Pownall TL, Bugera JL, et al. (2013) Zinc deficiency and taste perception in the elderly. Crit Rev Food Sci Nutr 53(3): 245-250.

21. Ananda Prasad (2003) Zinc deficiency. BMJ 326(409).

22. Zinc - Health Professionals. National Institutes of Health.

23. Zdilla M, Starkey L, Saling J (2015) A Taste-intensity Visual Analog Scale: An Improved Zinc Taste-test Protocol. Integr Med (Encinitas) 14(2): 34-38.

24. Roohani N, Hurrell R, Kelishadi R, Schulin R (2013) Zinc and its importance for human health: An integrative review. J Res Med Sci 18(2): 144-157.

25. Samaras D, Samaras N, Lang PO, Genton L, Frangos E, et al. (2013) Effects of widely used drugs on micronutrients: A story rarely told. Nutrition 29(4): 605-610.

26. Ranucci G, Dato FD, Spagnuolo M, Vajro P, Iorio R (2014) Zinc monotherapy is effective in Wilson's disease patients with mild liver disease diagnosed in childhood: a retrospective study. Orphanet J Rare Dis 9: 41.

27. Deshpande J, Joshi M, Giri P (2013) Zinc: The trace element of major importance in human nutrition and health. Int J Med Sci Public Health 2(1): 1-6.

28. Watts DL (1988) The Nutritional Relationships of Zinc. Journal of Orthomolecular Medicine 3(2): 63-67.

29. Masters DG, Keen CL, Lonnerdal B, Hurley LS (1983) Comparative aspects of dietary copper and zinc deficiencies in pregnant rats. J Nutr 113(7): 1448-1451.

30. Gomis Rüth FX (2003) Structural Aspects of the Metzincin Clan of Metalloendopeptidases. Mol Biotechnol 24(2): 157-202.

31. Massova IK, Kotra LP, Fridman R, Mobashery S (1998) Matrix metalloproteinases: structures, evolution, and diversification. FASEB J 12(12): 1075-1095.

32. Van Wart HE, Brickedal Hansen H (1990) The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. Proc Natl Acad Sci U S A 87(14): 5578-5582.

33. Nagase H, Visse R, Murphy G (2006) Structure and function of matrix metalloproteinases and TIMPs. Cardiovasc Res 69(3): 562-573.

34. Visse R, Nagase H (2003) Matrix Metalloproteinases and Tissue Inhibitors of Metalloproteinases: Structure, Function, and Biochemistry. Circ Res 92(8): 827-839.

35. Todorov G (2016) Matix Metalloproteinases. Smart Skin Care.

36. Chan Myers H, Mcalister D, Antonoplos P (1997) Natural bioburden levels detected on rigid lumened medical devices before and after cleaning. Am J Infect Control 25(6): 471-476.

37. Gibson D, Cullen B, Legerstee R, Harding K, Schultz G (2009) MMPs made easy. Wounds International 1(1): 1-6.

38. Høiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O (2010) Antibiotic resistance of bacterial biofilms. Int J Antimicrob Agents 35(4): 322-332.

39. Treatment of Pressure Ulcers - Clinical Practice Guideline Number 15 (1995) Rockville, MD: U.S. Dept. of Health and Human Services, Public Health Service, Agency for Health Care Policy and Research.

40. Thurlow LR, Hanke ML, Fritz T, Angle A, Aldrich A, et al. (2011) Staphylococcus aureus Biofilms Prevent Macrophage Phagocytosis and Attenuate Inflammation *In vivo*. J Immunol 186(11): 6585-6596.

41. Secor PR, James GA, Fleckman P, Olerud JE, Mcinnerney K, et al. (2011) Staphylococcus aureus Biofilm and Planktonic cultures differentially impact gene expression, mapk phosphorylation, and cytokine production in human keratinocytes. BMC Microbiology BMC Microbiol 11: 143.

42. Gruber K, Maywald M, Rosenkranz E, Haase H, Plumakers B, et al. (2013) Zinc deficiency adversely influences interleukin 4 and interleukin 6 signaling. J Biol Regul Homeost Agents 27(3): 661-671.

43. Valle J, Latasa C, Gil C, Toledo Arana A, Solano C, et al. (2012) Bap, a Biofilm Matrix Protein of Staphylococcus aureus Prevents Cellular Internalization through Binding to GP96 Host Receptor. PLoS Pathogens 8(8).

44. Gill S, Parks W (2008) Metalloproteinases and their inhibitors: Regulators of wound healing. Int J Biochem Cell Biol 40(6-7): 1334-1347.

45. Muller M, Trocme C, Morel F, Halimi S, Benhamou PY (2009) Increased Matrix Metalloproteinase-9 Predicts Poor Wound Healing in Diabetic Foot Ulcers: Response to Liu et al. Diabetes Care 32(11): e138-e138.

46. Smith AP (2003) The Role Of MMPs In Chronic Wound Edema. Podiatry Today 16(8): 22-26.

47. Kilpadi DV, Stechmiller JK, Childress B, Cowan L, Comerio M, et al. (2006) Composition of wound fluid from pressure ulcers treated with negative pressure wound therapy using VAC (R) therapy in home health or extended. Wounds 18(5): 119-128.

48. Gabriel A (2015) Wound Healing and Growth Factors. Medscape.

49. Barrientos S, Stojadinovic O, Golinko MS, Brem H, Tomic Canic M (2008) Growth factors and cytokines in wound healing. Wound Repair Regen 16(5): 585-601.

50. Rundhaug JE (2005) Matrix metalloproteinases and angiogenesis. J Cell Mol Med 9(2): 267-285.

51. Hattori N, Mochizuki S, Kishi K, Nakajima T, Takaishi H, et al. (2009) MMP-13 Plays a Role in Keratinocyte Migration, Angiogenesis, and Contraction in Mouse Skin Wound Healing. Am J Pathol 175(2): 533-546.

52. Philips N, Auler S, Hugo R, Gonzalez S (2011) Beneficial Regulation of Matrix Metalloproteinases for Skin Health. Enzyme Research 2011: 427285.

53. Lee H, Overall CM, Mcculloch CA, Sodek J (2006) A Critical Role for the Membrane-type 1 Matrix Metalloproteinase in Collagen Phagocytosis. Mol Biol Cell 17(11): 4812-4826.

54. Cytokines in Wound Healing: R&D Systems, 2002 catalog.