Micronutrient Status in Diabetic Patients with Foot Ulcers

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Objective: To explore the prevalence of micronutrient deficiencies in patients with diabetic foot ulcers and correlate this with foot disease severity and other clinical factors.

Approach: Prospective cohort study of diabetic patients with foot ulcers seen in multidisciplinary foot clinics across Adelaide or admitted to the Vascular Surgery Unit at the Royal Adelaide Hospital between February 2017 and September 2018. A total of 131 patients were included in the study. Plasma serum levels of vitamins A, C, D, and E, copper, zinc, and ferritin were measured. Demographic and clinical data, including BMI, smoking status, duration of diabetes, HbA1c, and WIfI score, were obtained.

Results: The most prevalent nutritional deficiency found was vitamin D affecting 55.7% of patients. Suboptimal levels of vitamin C affected 73% of patients, comprising marginal levels in 22.2% and deficient levels in 50.8%. Zinc deficiency, vitamin A deficiency, and low ferritin levels were present in 26.9%, 10.9%, and 5.9% of patients, respectively. There was no correlation between BMI, grip strength, duration of diabetes, HbA1c, or smoking status with micronutrient deficiency. Increased severity of diabetic foot disease was associated with lower vitamin C levels ($p = 0.02$).

Innovation: This study has demonstrated that the deficiency of micronutrients, especially vitamin D, vitamin C, zinc, and vitamin A, is common in diabetic patients with foot ulcers.

Conclusions: The prevalence of micronutrient deficiency is high in a diabetic population with foot ulcers/wounds. Special concerns regarding the high prevalence of vitamin C and zinc deficiency, given their roles in wound healing. Although further research needs to be performed to determine the clinical implications of our findings, micronutrient deficiency should be considered in diabetic patients with foot wounds.

Keywords: chronic wounds, diabetes, nutrition

INTRODUCTION

Diabetic foot complications carry a substantial physical, psychological, and financial burden for the patients and community. People who suffer from diabetes have a lifetime risk of nearly 25% of developing a foot ulcer, and more than 50% of patient ulcers will develop infection.[1] A history of foot ulcer is significantly associated with negative outcomes. Approximately 85% of all amputations in diabetic patients are preceded by foot ulceration,

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which subsequently deteriorates to foot infection or gangrene.[2]

Diabetic foot complications are recognized as the most common cause of nontraumatic lower limb amputation internationally. Worldwide it is estimated that every 20 s a lower limb amputation is performed as a consequence of diabetes.[3] The challenge to heal a diabetic foot ulcer is compounded by the high rate of reulceration once healed. Approximately 40% of patients have recurrence of an ulcer within 1 year after healing, almost 60% within 3 years, and 65% within 5 years.[3]

Multifactorial efforts should be made to give affected patients the best chance to heal foot ulcers. Traditionally, this has taken the form of local wound care, debridement, offloading, attention to infection, and revascularization if required. However, there are many other patient-related factors that are frequently overlooked that may influence wound healing (Table 1). One of the factors most commonly neglected is patient nutrition.

CLINICAL PROBLEM ADDRESSED

Wound healing is a complex, dynamic, and interactive process involving soluble mediators, blood cells, extracellular matrix, and parenchymal cells. Wound healing has three phases that overlap in time; inflammation, tissue formation, and tissue remodeling.[4] The relationship between nutrition and wound healing has been recognized for centuries. It is widely known that macronutrient malnutrition, especially protein, adversely affects wound healing. Equally important are micronutrients, which are critical components of cellular metabolism. A number of vitamins and minerals play a significant role in the immune system and wound healing, particularly important are vitamin C, vitamin A, and zinc.[5] Despite their roles, they are not routinely measured or monitored in clinical practice.

Table 1. *Factors considered to affect wound healing*

Poor or impaired perfusion
Infection
Smoking and alcoholism
Aging
Chronic diseases (*e.g.*, diabetes, chronic kidney disease, AIDS)
Malnutrition
Medication (*e.g.*, glucocorticoid steroids, nonsteroidal anti-inflammatory drugs, chemotherapy)
Obesity
Edema
Presence of foreign body
Venous insufficiency

The primary goal of this study was to assess the prevalence of vitamin and micronutrient deficiency in diabetic patients with foot ulcers seen either in an outpatient setting within multidisciplinary foot clinics, or inpatients admitted to our vascular service. The secondary goal was to correlate micronutrient levels with disease severity and other clinical factors, namely duration of diabetes, HbA1c levels, grip strength, and smoking status.

MATERIALS AND METHODS

This study is part of a major project assessing factors influencing outcomes in patients with diabetic foot disease. Ethics has been obtained from the Central Adelaide Local Health Network Ethics Committee, and written consent was obtained from all participants. Subjects consisted of patients seen at Multidisciplinary Foot Clinics at The Queen Elizabeth Hospital and Lyell McEwin Hospital, or admitted under the Vascular Surgery service at the Royal Adelaide Hospital, all within the Adelaide metro area of South Australia. Eligibility criteria included being diabetic, age ≥18 years, able to have follow-ups in Adelaide, and presence of foot ulcer(s).

A total of 131 patients were recruited for the study between February 2017 and September 2018. Plasma levels of vitamins A, C, D, and E; and copper, zinc, and ferritin were measured at recruitment. All the samples were venous blood taken by a phlebologist in the hospital or at an outpatient pathology collection center. Specimens were handled and transported as per collection guide and all processed by SA Pathology. Table 2 shows reference levels used for analysis. Demographic information and clinical data were pro-

Table 2. *Reference levels of micronutrients*

Vitamin A	<0.7 μmol/L	Deficient
	≥0.7 μmol/L	Nondeficient
Vitamin C	<11.4 μmol/L	Deficient
	11.4–22.7 μmol/L	Marginal
	>22.7 μmol/L	Adequate
Vitamin D	<60 nmol/L	Deficient
	≥60 nmol/L	Nondeficient
Vitamin E	<12 μmol/L	Deficient
	≥12 μmol/L	Nondeficient
Zinc	<9 μmol/L	Deficient
	≥9 μmol/L	Nondeficient
Copper	<10 μmol/L	Deficient
	≥10 μmol/L	Nondeficient
Ferritin	<30 μg/L	Deficient
	≥30 μg/L	Nondeficient[a]

[a]Ferritin is an acute-phase reactant and significantly higher cutoff levels for ferritin are used to define iron deficiency accompanied by inflammation.

Table 3. *Summary of participant characteristics*

Variable	*Level*	*Number*	*Percent*
Gender	Female	27	20.6
	Male	104	79.4
Smoking status	Current	31	23.7
	Exsmoker	62	47.3
	Never	38	29
WIfI category	1	25	19.1
	2	32	24.4
	3	33	25.2
	4	41	31.3
Variable		*Mean*	*SD*
Age (years)		66.3	13.1
Duration of diabetes (years)		16.4	10.7
HbA1c (%)		8.8	4.4
BMI		29.4	6.1
Grip strength (kg)		29.1	9.6

spectively obtained during patient assessment, including age, gender, weight, height, BMI, and smoking status. In addition, grip strength and WIfI score were also recorded. Grip strength is a measurement of muscle function as an indicator of functional as well as nutritional status.[6] The WIfI is a validated classification that stratifies patients with threatened lower extremity, including patients with diabetic foot ulcers, based on three major factors that impact amputation risk and clinical management: Wound, Ischemia, and foot Infection.[7]

The association between the nutrients most commonly cited as important for wound healing, namely vitamin C, vitamin A, and zinc, and smoking status, grip strength, duration of diabetes (since diagnosis), HbA1c levels, and burden of diabetic foot disease assessed by WIfI, was assessed.

Statistics

Continuous measures are summarized as means with standard deviations and medians with interquartile range. Categorical measures are presented as counts and percentages. Associations between nutrient deficiencies and continuous predictors were determined using the Kruskal–Wallis test (vitamin C deficiency) or Wilcoxon test (remaining nutrients) as appropriate. The associations between nutrient deficiency and categorical predictors were assessed using Pearson's chi-square or Fisher's exact test as appropriate. There was no formal statistical assessment for vitamin E or copper deficiencies as all patients were in the normal range for these nutrients.

RESULTS

One hundred thirty-one patients were enrolled in the study. The characteristics of the participants are shown in Table 3. Figure 1 summarizes the prevalence of micronutrient deficiencies. The elements most frequently found to be deficient, in descending order, were vitamin D, vitamin C, zinc, ferritin, and vitamin A. None of the patients had low levels of vitamin E or copper.

Twenty-seven percent of patients had normal levels of vitamin C. The remainder had suboptimum levels with just over half of all the patients having low or no measurable plasma levels of this vitamin (Fig. 2).

There was no association between the duration of diabetes, HbA1c levels, grip strength, smoking habits, and BMI and levels of vitamin A, C, and

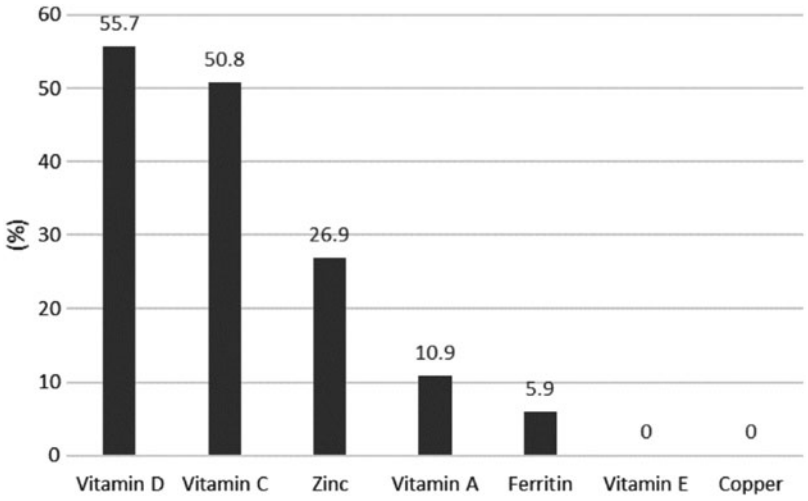

Figure 1. Percentage of participants with vitamin and mineral deficiencies.

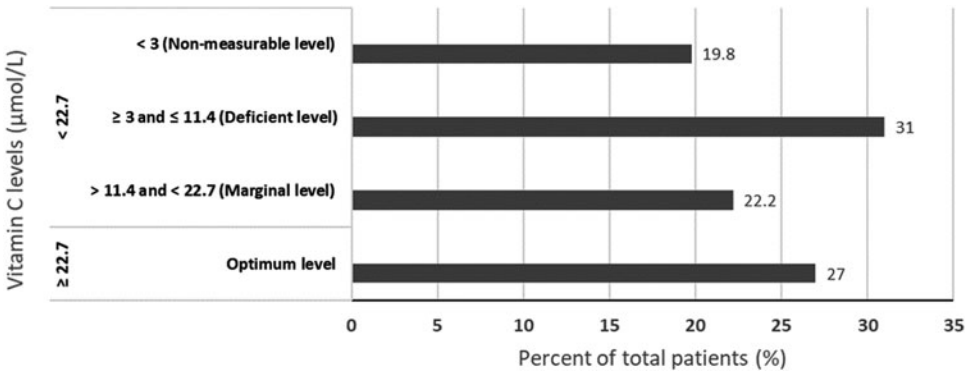

Figure 2. Vitamin C levels.

zinc. Patients with a higher burden of foot disease as assessed by the WIfI score had lower levels of vitamin C ($p = 0.02$) and higher ferritin level ($p = 0.004$). Lower grip strength and smoking habit were associated with lower vitamin D levels ($p = 0.02$ and 0.01, respectively) (Table 4).

DISCUSSION

Vitamins and minerals are required in small amounts, yet they are critical to the cellular metabolism, including the wound healing process. This study has demonstrated that micronutrient deficiencies are very common in diabetic patients with foot ulcers/wound.

Vitamin D deficiency was the most common deficiency detected, and its prevalence is consistent with previous reports.[8] This was expected as vitamin D deficiency is recognized as a global public health problem, usually related to sunscreen use and sun avoidance behaviors.[9] Diabetic patients with foot ulcers may be at particular risk of vitamin D deficiency due to reduced level of physical activity, which is often considered a surrogate for the amount of time spent outdoors and therefore sun exposure. Vitamin D is well recognized for its role in the bone homeostasis. However, vitamin D signaling has also many extraskeletal effects. These include regulation of cell proliferation, immune and muscle function, skin differentiation, and reproduction, as well as vascular and metabolic properties.[10] Despite the abundance of preclinical data regarding vitamin D and skin cell interaction, there is no good-quality evidence to support a substantial role in wound healing. The association between reduced levels of vitamin D and smoking habits is consistent with previous reports, however, the mechanism of this association is unclear.[11,12] Vitamin D deficiency was associated with lower grip strength. This is can be explained by the fact that lower muscular strength is one of the aspects of sarcopenia and frailty that commonly accompany aging. It is well known that geriatric patients are at higher risk of vitamin D deficiency due to a lower sunshine exposure and a reduced capacity of the older skin to synthesize vitamin D under the influence of UV light.[13]

The prevalence of deficiencies in vitamin C, zinc, and vitamin A was higher than anticipated, and concerning, given the pivotal roles these nutrients play in wound healing. Iron status was assessed by ferritin levels, which is a marker of iron stores in the body. Six percent of the patients had ferritin less than 30 μg/L and were considered deficient. However, the prevalence of iron deficiency is likely higher than identified by our study. Ferritin is an acute-phase reactant, and a significantly higher cutoff level for ferritin is used to define iron deficiency accompanied by inflammation.[14] This explains why there was a positive correlation between ferritin levels and WIfI score, for which one of the components is the presence and severity of infection. None of the study patients had biochemical deficiency of copper or vitamin E. Deficiency of copper and vitamin E in adults is extremely rare

Table 4. *Association between micronutrients and clinical parameters*

	Smoking Status (p Value)[a]	*Grip Strength (p Value)*	*Duration of Diabetes (p Value)*	*HbA1c (p Value)*	*WIfI Score*[b] *(p Value)*
Vitamin C	0.13	0.77	0.21	0.70	**0.02**
Vitamin A	1.0	0.39	0.46	0.82	0.06
Zinc	0.30	0.87	0.98	0.41	0.05
Ferritin	0.34	0.63	0.78	0.47	**0.004**
Vitamin D	**0.02**	**0.01**	0.21	0.19	0.73

Bold values are statistically significant.

There was no formal statistical assessment for vitamin E or copper deficiencies as all patients were in the normal range for these nutrients.

[a]Assessed as current smoker or nonsmoker.

[b]Fisher's exact test (Pearson's chi-square test otherwise).

and generally related to physiological abnormalities such as the malabsorption syndrome. It is not anticipated that deficiency of these elements will be a consequence of simple reduced intake.[15,16]

Vitamin C (ascorbic acid) is an important water-soluble vitamin, essential for collagen, carnitine, and neurotransmitter biosynthesis.[17] Vitamin C is a cofactor for prolyl and lysyl hydroxylase, two essential enzymes in the collagen biosynthesis pathway, and as such ensures the maintenance of normal mature collagen networks in humans. Hydroxyproline serves to stabilize the triple helix, and hydroxylysine is necessary for the formation of the intermolecular crosslinks in collagen, which is critical to the biological functions of this protein.[18] This failure of collagen synthesis in deficiency of vitamin C leads to the manifestations characteristic of scurvy.[17,18] An interesting experiment that validates the role of vitamin C in skin hemostasis was conducted in 1939 by John Crandon, a second-year surgical resident at Boston City Hospital. Crandon commenced a vitamin C-free diet and published a detailed description of the dramatic changes he experienced during this period. Notably, at about 6 months into the experiment, his appendectomy scar from years ago began to disintegrate. In addition, a back incision, performed as part of the experiment, failed to heal and a biopsy demonstrated lack of "intercellular substance." Following the administration of IV vitamin C, the wounds rapidly healed.[19]

Only 27% of patients in the present study had optimum levels of vitamin C, 22% had marginal levels, and 51% were deficient. Approximately 1 in 5 patients had nonmeasurable levels. These findings were surprising, given this vitamin is found widely in fruits and vegetables. Although low plasma ascorbic acid levels do not necessarily indicate scurvy, serum levels have a linear relationship with vitamin C intake and clinical cases of scurvy always have low or no measurable plasma ascorbic acid.[20]

Many common conditions are thought to result in pro-oxidant states that contribute to low vitamin C levels, including cigarette smoking, diabetes, and acute illnesses.[21,22] In this study, there was no correlation between vitamin C levels and smoking history, diabetic control (measured by HbA1c), or duration of diabetes. Patients with more advanced foot disease burden assessed by WIfI classification had lower vitamin C levels. This could be related to the effect of inflammation and acute illness on plasma vitamin C levels or could be related to lower vitamin intake in the group with more advanced disease. It is known that diabetic foot disease has a profound impact on patients' quality of life. Diabetic foot ulceration is associated with restricted mobility, social isolation, and reduced self-esteem.[23] It can be assumed that patients with more severe disease burden assessed by the WIfI score would experience more drastic impact on mobility and social life and therefore would be less likely to have a diet rich in fresh fruit and vegetables.

It is well documented that patients with scurvy present with weakening of connective tissues and poor wound healing.[17,18] However, there are no definitive data showing that increasing the vitamin C concentration directly enhances its biochemical or molecular function in human tissues, or that higher vitamin C levels confer a wound-healing benefit in patients without scurvy. There is lack of evidence from well-designed studies to support the theory that vitamin C supplementation above the recommended daily allowances improves wound healing. However, it does make sense to treat patients with reduced levels, especially in the presence of chronic ulcers or large wounds.

Zinc is the second-most abundant trace element in the human body after iron[24] and its main sources are animal products and seafood. It is an essential trace element crucial for the function of more than 300 enzymes and it is important for cellular processes such as cell division and apoptosis.[25] Zinc plays an important role in wound healing as it serves as a cofactor in numerous transcription factors and enzyme systems, including zinc-dependent matrix metalloproteinases. Matrix metalloproteinases are a group of calcium-dependent zinc-containing enzymes that are involved in the degradation of extracellular matrix (ECM). Metalloproteinases and their inhibitors are essential for the regulation of ECM degradation and deposition during wound repair.[26] In this study, 27% of diabetic patients with foot ulcers had low levels of this mineral. There was no correlation of zinc levels with diabetic duration and control, grip strength, or smoking status. The patients with more advanced foot disease tended to have lower zinc levels and this correlation nearly reached statistical significance ($p = 0.05$). It is important to note that serum zinc concentrations may not fully reflect the physiological zinc status in an individual and factors such as inflammation may affect plasma levels.

The role of oral supplementation of zinc in wound healing is controversial. A frequently cited randomized controlled trial (RCT) from 1967 demonstrated decreased wound healing time by 43% in patients with pilonidal sinus wounds receiving oral zinc sulfate supplements.[27] Serum zinc concentrations in participants in this study were not measured. A more recent RCT with patients with diabetic foot ulcers demonstrated a statistically

significant improvement in wound healing following 12 weeks of supplementation of zinc.[28] However, both studies had small sample size and did not select patients based on zinc parameters.

Vitamin A is an essential, dietary, fat-soluble vitamin that has multiple functions, including an important role in wound healing. It is involved in epithelial differentiation and proliferation, stimulation of angiogenesis, collagen synthesis, and fibroplasia. Vitamin A also has a unique ability to reverse the inhibitory effects of glucocorticosteroids on wound healing.[29] Vitamin A is available in the human diet in two forms: preformed vitamin A, found in food from animal sources, and provitamin A carotenoids, such as beta-carotene present in fruits and vegetables. Most of the vitamin A in the body is stored in the liver, and the plasma contains only $\sim$1% of the total body reserve. Levels can be reduced in the setting of inflammation and liver disease. Vitamin A deficiency is a public health problem in low-income countries, especially in Africa and Southeast Asia, where it is the leading cause of preventable blindness. Although vitamin A deficiency is considered rare in developed countries, 11% of the diabetic patients with foot ulcers included in this study had low retinol levels (<0.7 μmol/L) and one patient had severe deficiency (<0.35 μmol/L).

The reason for low vitamin levels in the studied patients is likely multifactorial, with poor intake playing a major role. To ensure adequate micronutrient intake, a diverse diet is required. Micronutrient-rich foods include fruits, vegetables, meat, dairy, seafood, nuts, and seeds. Maintaining a rich and diverse diet may be particularly difficult for low-income households as it may be less affordable than a more energy-dense diet. It is known that diabetic ulcers are associated with reduced quality of life, decrease mobility, and social isolation.[23] This potentially impairs the ability of affected patients to go shopping frequently for fresh food. This is particularly important for vitamin C, which is found in many fruits and vegetables. Vitamin C levels in food depend on transport, storage, and cooking practices.[30] In addition, there may be insufficient education about nutrition, poor cooking skills, and a heathy diet rich in vegetables and fruits may also be perceived as not palatable or boring.

This study's primary aim was to assess the prevalence of micronutrient deficiency in diabetic patients with foot ulcers. We acknowledge that there are many potential confounders that may influence the micronutrient levels and would be ideal to have a detailed nutritional history from the patients, including history of malabsorption syndromes, dietary intake, and supplement use. It would be also interesting to compare the results with control groups of individuals without diabetes and a group of diabetic patients without history of foot ulcers. The sample size of this study was large, and the selection criteria were broad to include a whole heterogeneity of diabetic population with foot ulcers. Although further research needs to be performed to determine the clinical implications of our findings, vitamin and mineral deficiency should be considered in all diabetic patients with foot wounds. Foot ulcers are a common and challenging complication of diabetes and constitute a substantial burden for these patients. The management should be based on intensive multimodality therapy aiming to achieve wound healing, reduce risk of reulceration, and an improved quality of life.

INNOVATION

This study has demonstrated that the prevalence of micronutrient deficiency, especially vitamin D, vitamin C, zinc, and vitamin A, is high in diabetic patients with foot ulcers. Currently, there is no good-quality evidence to support micronutrient supplementation to improve wound healing and this study did not assess clinical correlation with outcomes. However, in light of the physiological role of some micronutrients, especially vitamins C and A, and zinc, the complexity of the diabetic foot disease, and the high prevalence of micronutrient deficiency found in this study, we suggest assessing the levels of these vitamins and minerals in patients with diabetic foot ulcers and considering supplementary treatment if deficiency is found.

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KEY FINDINGS

- Suboptimal levels of vitamin C affected 73% of diabetic patients with foot ulcers, comprising marginal levels in 22.2% and deficient levels in 50.8%.
- Zinc deficiency was found in ~27% of the patients.
- Vitamin A deficiency was present in ~11% of the patients.
- Although further research needs to be performed to determine the clinical implications of our findings, micronutrient deficiency should be considered in diabetic patients with foot wounds.

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Abbreviations and Acronyms

ECM = extracellular matrix
RCT = randomized controlled trial