Wound tissue concentration of essential elements and healing of leg ulcers in Sri Lankan patients: is there a link?

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ABSTRACT

Wounds are likely to have existed ever since mankind has existed. Wound healing is an enormously complicated process and the actual scientific mechanisms and events that take place during healing are far more complex and dynamic than might be imagined. Essential elements, especially trace elements are believed to be pivotal to the wound healing process. Their involvement in tissue regeneration and repair appears to be wide ranging and their deficiencies have been reported to impair the healing process. However, further research is required to establish the involvement of trace elements and their specific species in the wound healing process.

Thus assessments of trace element levels in wound tissues using new, reliable, verified and validated technologies could be beneficial for trace element based wound healing. Here, is an attempt made to assess the link between trace element concentrations and healing processes of chronic and acute wounds. Wound tissue samples from 58 chronic leg ulcers and 50 acute wounds were analysed for concentrations of Fe, Zn, Cu, Mn, Ca, Sn, Cr, Cd and Pb using atomic absorption spectrophotometer. Blood samples were also collected from the same patients and analysed in the same manner. All the data were tested for normality by Ryan–Joiner normality test ($\alpha = 0.05$) and one-way ANOVA was done for the normally distributed data.

The results showed that the concentration of Ca, Zn, Cu and Mn were similar in both acute and chronic wounds. Apart from Ca, which showed elevated concentrations, other metal concentrations are either similar or lower than the analysed concentrations in blood. It appears that there is a significant difference in the concentrations of iron accumulated in the tissues of chronic and acute wounds.

The concentrations of Sn, Cr, Cd and Pb, which are considered as toxic metals, were not present in detectable levels with the graphite furnace atomic absorption spectrophotometry in both types of wound tissues.

Keywords: essential elements, wounds, healing, zinc, copper

1. INTRODUCTION

Most individuals have suffered a wound during their life time. Slow, sometimes non-healing persistent wounds are termed 'chronic wounds', because their underlying aetiology leads to long, complicated, painful process, which reduces the quality of life for an individual and places a significant burden upon the financial resources of the health care economy (Jones and Williams, 2002).

Normal healing comprises a series of perfectly coordinated events assisted by the presence and action of specific chemical and cellular mediators. In order for a wound to heal, it has to be fed either using the body's own resources, which in many cases are inadequate because of vascular damage through using supplements which may be applied directly at the site of the wound, rather than rely on supplementation that is dependent on the circulation (Prasad, 1976).

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The roles of essential trace elements in tissue repair and regeneration are still unclear, as a result of the extremely complex biochemical and cellular reactions involved, and also the presence of myriad of many trace elements, individual chemical and cellular species present in the tissue; these in total comprise the localized healing matrix (Williams, 1996).

Many trace elements play multi-factorial roles in the healing process. Certain trace elements may influence the bioavailability and uptake of others which may be beneficial or hinder the healing process (Liyanage, 1996; Liyanage *et al.*, 1995). The careful balancing of trace metals required for efficient healing and the imbalances in tissue ions are believed to be the potential causes of delayed or refractory healing (Liyanage *et al.*, 1996). Hence, it is not unreasonable to suggest that any imbalance of trace element species at the wound site may alter the normal healing process, and thus it is hypothesized that there may be a significant association between wound tissue essential element levels and wound healing.

To examine this, a quantitative assessment of essential metals and heavy metals in the tissue of chronic and acute wounds and blood was carried out.

2. MATERIALS AND METHODS

Fresh tissue samples (by 2 mm biopsy punch) (58 from chronic wounds, 34 male leg ulcers and 24 female leg ulcers; 50 from acute wounds, 29 male samples, 21 female samples) were obtained from the National Hospital of Sri Lanka. At the same time, blood samples were collected from the same patients to compare the metal concentrations for any variations.

Samples of chronic wound tissue were collected from the sites of wounds which were clinically regarded as different types of chronic leg ulcers (16 venous ulcers, 33 diabetes cases, and nine pressure ulcers). Samples of acute wound tissue were also collected from the sites of fresh wounds from surgical theatres which were apparently healing normally and they were regarded as the fresh tissue samples. All the samples were stored in polypropylene vials at freezing conditions until analysed (Taylor *et al.*, 1998).

Samples of wound tissues from both chronic and acute were deprotinized (acid digested) in a mixture of acids having a ratio of concentrated nitric acid to concentrated perchloric acid 4 : 1. This mixture was boiled and the liquid was evaporated until a white

Table 1 Mean concentration of iron in wound tissues and blood of the relevant patient (μmol/g) ($n = 3$)

Acute wound tissue	Blood	Chronic wound tissue	Blood
1.200	4.116	1.502	3.759
0.936	4.271	2.294	2.840
1.429	3.509	2.169	4.225
1.064	3.184	2.795	4.258
1.469	3.693	1.810	4.409
1.068	3.546	1.101	3.188
0.655	3.660	1.323	3.660
1.138	3.999	2.736	2.781
0.687	3.649	1.446	2.496
1.373	3.581	2.255	3.581
0.609	3.227	1.759	3.227
0.433	2.547	0.653	2.798
0.819	2.899	2.107	2.510
1.396	4.057	1.553	3.843
1.052	3.908	1.067	2.946
0.945	3.566	1.260	2.278
1.563	3.216	1.533	2.508
0.631	3.648	0.946	3.258
0.786	2.519	1.277	2.897
0.506	3.647	0.828	4.268
0.374	4.278	1.408	2.847
0.417	4.641	0.592	3.210
0.112	3.670	0.159	3.882
1.080	3.401	0.553	2.472
1.055	3.913	0.706	2.782
1.417	2.855	0.991	3.943
1.422	3.528	1.648	3.235
1.453	4.194	1.206	2.830
1.068	3.288	0.091	1.834
0.416	4.387	0.630	2.627
1.359	3.981	1.182	3.699
0.431	4.285	0.427	2.862
1.385	3.581	0.794	4.001
0.361	4.301	1.360	2.891
0.392	3.513	0.730	4.005
1.038	3.583	1.585	2.705
1.396	4.200	1.620	3.189
0.694	3.662	1.546	4.240
0.863	3.352	1.452	3.188
1.426	2.865	1.847	2.790
0.631	3.616	1.735	3.585
1.062	3.255	1.552	3.759
0.506	3.911	1.936	2.840
0.374	4.635	2.169	4.225
0.417	3.926	2.651	3.900
0.112	4.028	1.931	4.051
0.506	4.268	1.101	3.903
0.374	4.492	1.234	2.945
0.417	3.648	2.370	2.411
0.112	3.981	1.088	3.211
		2.790	3.227
		0.799	3.611
		0.034	3.174
		0.883	3.941
		1.396	3.119
		0.337	3.306
		1.405	3.914
		0.379	4.072

Table 2 Mean concentration of calcium in wound tissues and blood of the relevant patient (μmol/g) ($n = 3$)

Acute wound tissue	Blood	Chronic wound tissue	Blood
5.130	1.955	5.343	1.597
7.923	1.447	4.977	0.999
5.941	1.195	5.763	1.472
7.033	1.490	3.457	1.430
6.422	1.479	6.860	0.980
5.524	1.422	3.982	0.923
5.266	0.953	5.515	0.953
4.608	0.751	4.913	0.751
7.307	0.867	3.148	0.867
5.458	0.889	4.457	0.889
3.534	0.842	5.775	0.842
5.122	0.994	5.433	0.994
3.011	1.751	5.636	1.751
4.923	0.961	3.428	0.961
6.456	1.219	5.763	1.219
3.942	1.036	6.771	0.538
6.482	0.926	0.742	0.926
3.359	0.999	6.624	0.999
5.822	1.476	7.554	1.476
6.504	1.163	5.941	1.163
5.254	0.974	6.012	0.758
5.610	1.098	6.223	1.098
7.647	0.900	5.444	0.525
5.045	1.597	5.416	1.023
7.957	1.448	4.558	1.400
5.924	1.472	7.300	0.961
7.002	1.430	4.481	0.920
6.383	0.980	3.785	0.508
5.474	1.422	6.100	0.905
5.264	0.953	4.008	0.691
4.685	1.472	4.582	0.911
7.173	0.867	5.951	0.846
5.474	0.889	3.576	0.987
3.532	1.341	5.078	1.247
5.005	0.994	7.940	0.633
3.023	1.751	5.801	1.292
4.921	0.961	6.986	0.952
6.455	1.219	6.367	1.409
3.953	0.952	5.474	1.038
6.479	0.926	5.281	1.296
3.486	0.999	4.683	1.647
5.803	1.476	7.169	0.624
6.520	1.163	5.483	1.636
5.247	0.758	3.532	0.999
5.625	1.098	5.008	1.479
7.649	0.974	3.011	1.131
6.524	1.163	4.923	0.746
5.237	1.257	6.456	0.957
5.625	1.098	3.942	0.943
7.638	0.924	6.482	0.574
		3.484	0.835
		5.797	0.987
		6.509	0.796
		5.237	0.648
		5.627	1.921
		7.647	0.595
		4.960	1.169
		5.992	0.637

Table 3 Mean concentration of zinc in wound tissues and blood of the relevant patient (μmol/g) ($n = 3$)

Acute wound tissue	Blood	Chronic wound tissue	Blood
1.056	1.865	0.439	1.817
0.614	1.581	0.228	1.440
0.614	0.643	1.119	0.925
0.308	0.968	0.289	0.810
0.919	1.588	0.599	1.506
1.225	1.229	0.550	1.557
0.919	1.562	0.869	1.250
0.309	0.969	0.302	0.511
0.920	0.670	0.234	1.197
0.614	0.921	0.440	0.617
0.275	0.893	0.279	1.501
0.308	1.201	0.103	1.829
0.615	0.856	0.389	1.161
0.919	1.595	0.299	0.708
0.277	0.775	0.964	0.612
0.614	0.813	0.152	0.511
0.614	0.817	0.576	0.526
0.309	1.362	0.555	1.617
1.226	0.463	0.797	0.769
0.615	1.069	0.454	0.899
0.612	0.782	0.792	1.449
0.309	1.396	0.660	1.139
1.226	0.989	0.796	0.919
1.056	1.853	0.209	0.901
0.614	1.581	1.156	0.607
0.614	0.641	0.327	0.630
0.308	1.124	0.414	0.596
0.919	1.771	0.838	0.728
1.225	1.232	0.968	0.627
0.919	1.617	0.274	0.682
0.309	1.060	0.631	0.903
0.979	0.915	0.545	0.455
0.674	0.951	0.869	0.896
0.581	0.890	0.602	0.596
0.308	0.978	0.234	0.836
0.615	0.917	0.503	0.919
0.919	1.791	0.242	0.750
0.583	0.791	0.968	1.228
0.623	0.813	0.314	0.440
0.614	0.664	0.611	0.886
0.318	1.361	1.081	0.457
1.237	0.494	0.888	0.789
0.615	1.027	0.631	0.803
0.613	0.874	1.194	1.182
0.309	1.304	0.592	0.836
1.229	0.720	0.467	0.863
0.615	1.048	1.196	1.276
0.611	0.813	0.643	1.184
0.309	1.304	0.885	0.919
1.226	0.699	0.463	0.855
		0.269	0.607
		1.277	0.889
		0.580	0.596
		0.097	0.807
		0.195	0.827
		0.581	0.710
		0.621	0.641
		1.493	0.955

Table 4 Mean concentration of copper in wound tissues and blood of the relevant patient (μmol/g) ($n = 3$)

Acute wound tissue	Blood	Chronic wound tissue	Blood
0.031	0.050	0.040	0.040
0.032	0.074	0.041	0.077
0.027	0.035	0.045	0.032
0.033	0.063	0.032	0.069
0.023	0.048	0.033	0.051
0.019	0.058	0.040	0.062
0.029	0.076	0.031	0.073
0.023	0.088	0.046	0.087
0.020	0.061	0.026	0.061
0.021	0.068	0.004	0.067
0.033	0.102	0.015	0.102
0.034	0.070	0.043	0.039
0.034	0.077	0.034	0.080
0.029	0.045	0.059	0.045
0.034	0.071	0.024	0.074
0.024	0.059	0.032	0.034
0.032	0.050	0.031	0.056
0.032	0.063	0.023	0.066
0.023	0.067	0.019	0.036
0.029	0.060	0.029	0.060
0.036	0.067	0.023	0.067
0.023	0.044	0.020	0.032
0.024	0.075	0.021	0.087
0.031	0.040	0.033	0.046
0.032	0.077	0.034	0.039
0.027	0.032	0.034	0.074
0.033	0.069	0.029	0.058
0.023	0.051	0.034	0.093
0.019	0.062	0.024	0.071
0.029	0.073	0.032	0.049
0.023	0.087	0.032	0.047
0.020	0.061	0.023	0.050
0.021	0.067	0.029	0.052
0.033	0.102	0.036	0.066
0.034	0.039	0.023	0.062
0.034	0.080	0.024	0.035
0.029	0.045	0.014	0.049
0.034	0.074	0.037	0.037
0.024	0.034	0.027	0.069
0.032	0.056	0.037	0.041
0.032	0.066	0.032	0.041
0.023	0.036	0.024	0.050
0.029	0.060	0.054	0.045
0.036	0.067	0.029	0.041
0.023	0.032	0.050	0.050
0.024	0.087	0.023	0.046
0.029	0.060	0.024	0.064
0.036	0.067	0.008	0.039
0.023	0.032	0.030	0.060
0.024	0.087	0.014	0.035
		0.038	0.045
		0.032	0.034
		0.010	0.067
		0.020	0.066
		0.031	0.052
		0.032	0.050
		0.027	0.047
		0.033	0.066

Table 5 Mean concentration of Manganese in wound tissues and blood of the relevant patient (μmol/g) ($n = 3$)

Acute wound tissue	Blood	Chronic wound tissue	Blood
0.007	0.055	0.010	0.055
0.008	0.056	0.005	0.056
0.006	0.045	0.007	0.045
0.006	0.064	0.006	0.064
0.008	0.051	0.005	0.051
0.004	0.066	0.006	0.066
0.007	0.065	0.006	0.065
0.005	0.049	0.007	0.049
0.006	0.064	0.004	0.064
0.007	0.070	0.005	0.070
0.008	0.062	0.007	0.062
0.007	0.062	0.006	0.062
0.007	0.058	0.006	0.058
0.004	0.047	0.005	0.047
0.005	0.046	0.004	0.046
0.010	0.075	0.009	0.075
0.005	0.038	0.007	0.038
0.007	0.071	0.005	0.071
0.006	0.043	0.005	0.043
0.005	0.078	0.004	0.078
0.006	0.076	0.009	0.076
0.006	0.062	0.004	0.062
0.007	0.071	0.004	0.071
0.007	0.055	0.007	0.045
0.008	0.056	0.008	0.081
0.006	0.045	0.006	0.049
0.006	0.064	0.004	0.058
0.008	0.051	0.007	0.067
0.004	0.066	0.007	0.057
0.007	0.065	0.005	0.068
0.005	0.049	0.007	0.084
0.006	0.064	0.006	0.080
0.007	0.070	0.006	0.064
0.008	0.062	0.006	0.051
0.007	0.062	0.007	0.066
0.007	0.058	0.007	0.065
0.004	0.047	0.005	0.071
0.005	0.046	0.006	0.064
0.010	0.075	0.007	0.070
0.005	0.038	0.008	0.062
0.007	0.071	0.006	0.062
0.006	0.043	0.007	0.071
0.005	0.078	0.005	0.047
0.006	0.076	0.007	0.046
0.006	0.062	0.008	0.075
0.007	0.071	0.006	0.038
0.005	0.078	0.006	0.064
0.006	0.076	0.008	0.043
0.006	0.062	0.004	0.056
0.007	0.071	0.007	0.040
		0.005	0.073
		0.006	0.062
		0.007	0.045
		0.008	0.041
		0.007	0.049
		0.007	0.058
		0.004	0.053
		0.005	0.046

Table 6 Total mean concentration of elements present in tissues of chronic and acute wounds and blood

Element	Mean concentration in chronic wounds (μmol/g) ± SD 3.71 ± ($n = 58$)	Mean concentration in blood (μmol/g) ± SD ($n = 58$)	Mean concentration in acute wounds (μmol/g) ± SD ($n = 50$)	Mean concentration in blood (μmol/g) ± SD ($n = 50$)
Fe	1.36 ± 0.6	3.31 ± 0.7	0.85 ± 0.43	3.71 ± 0.5
Ca	5.30 ± 1.34	1.02 ± 0.04	5.63 ± 1.3	1.17 ± 0.3
Zn	0.60 ± 0.33	0.92 ± 0.35	0.70 ± 0.31	1.08 ± 0.4
Cu	0.03 ± 0.01	0.06 ± 0.02	0.028 ± 0.01	0.06 ± 0.02
Mn	0.006 ± 0.01	0.06 ± 0.02	0.006 ± 0.01	0.06 ± 0.01

solid residue was obtained. This residue was then dissolved in 25 cm^3 of doubled distilled deionised water (Howells *et al*; 1995). These digested samples were analysed for Fe, Zn, Cu, Ca, Mn, Sn, Cr, Cd and Pb using flame and graphite furnace atomic absorption spectrophotometer (AAS) GBC Aventa 932 plus (Liyanage *et al.*, 1995).

These data were analysed using the Minitab Ver.13.30 on Windows statistical data analysis software package. All the data were tested for normality by Ryan–Joiner normality test ($\alpha = 0.05$). When the data are found to be normally distributed One-way ANOVA tests were carried out.

3. RESULTS AND DISCUSSION

The concentration variations of Fe Ca, Zn, Cu and Mn (in μmol/g) of chronic and acute wound tissue with blood samples measured in 108 patients are shown in Tables 1–5 [Fe (Table 1), Ca (Table 2), Zn (Table 3), Cu (Table 4), Mn (Table 5). These Tables list the mean concentration of three replicate analyses on each sample.

The total means of the analysed concentration of each element in chronic and acute wound tissues with the mean elemental concentration of blood are illustration in the Table 6.

In the case of iron, the mean concentration of analysed chronic wound tissue was 1.36 μmol/g while the acute tissue sample was 0.85 μmol/g. Statistically there is a significant difference between two types of wounds ($P = 0.0001$) after ANOVA ($\alpha = 0.05$). It can clearly be seen that although the blood iron levels were always higher than the levels in tissues of both types of wounds, iron is accumulated in chronic wound tissues.

Calcium concentrations of analysed chronic tissue samples and acute tissue samples were 5.30 μmol/g and 5.63 μmol/g respectively. ANOVA ($\alpha = 0.05$) proved that there is no statistically proven difference between these two types of wounds ($P = 0.228$). Interestingly the calcium levels in all (in both types of wounds) wound tissues were significantly higher than the calcium in the blood of the same patient. Furthermore, calcium is heavily accumulated in the healing wound tissues.

The mean zinc concentration in chronic wound tissue was 0.60 μmol/g and the mean concentration of Zn in analysed acute wound tissue was 0.70 μmol/g. ANOVA ($\alpha = 0.05$) showed that there was no statistically proven difference between these two types of tissues ($P = 0.158$). In many acute wound tissues and some chronic wound tissues, the zinc levels in blood and tissues were very similar. This can be due to the fact that tissues need zinc for the healing process and blood has a constant supply of zinc for those tissues (Lansdown, 1996).

When considering the mean concentrations of copper in the analysed two types of wound tissues (0.03 μmol/g in chronic and 0.028 μmol/g in acute), ANOVA ($\alpha = 0.05$) showed that there is no statistically significant difference between them ($P = 0.297$). The concentration of copper in blood was always higher than the copper in chronic and acute wound tissues.

The concentration values for the manganese have not shown any significant variation in tissues of two different types ($P = 0.518$) after ANOVA ($\alpha = 0.05$), since the mean concentration of Mn in analysed chronic wound tissue is 0.006 μmol/g and the mean concentration of Mn in analysed acute wound tissue is 0.006 μmol/g. The Mn concentrations in the wound tissues of both types were significantly lower than the blood Mn levels.

The results show that the behaviours of Ca, Zn, Cu and Mn on wound healing were similar in both the acute and chronic wounds. However, Ca is heavily accumulated in healing wounds in both types. But in the case of iron, as statistical analyses prove, there is a significant variation in the behaviour between the two different types of wounds.

The concentrations of Sn, Cr, Cd and Pb were not detectable in both types of wound tissues even in graphite furnace AAS which can detect the metals in parts per billon levels.

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