

Full Length Research Paper

The role of trace elements in wagner classified diabetic patients

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Wagner's classification is the most widely used grading system in diabetic foot infections. The aim of this study was to evaluate the role of the trace elements in diabetic foot infected patients graded with Wagner's classification. This prospective study was carried out on 50 diabetic patients with variable severity of foot infections and 50 age and sex matched healthy subjects in Dicle University, Turkey, between October 2006 and November 2008. Diabetic foot infections were graded according to Wagner classification and deep tissue cultures were performed from all diabetic foot infected patients. The atomic absorption spectrophotometer was used in the measurement of trace elements in the serum. serum zinc and copper levels were significantly elevated in patients with Wagner 3 and 4 compared to Wagner 2 and 1 graded patients and healthy controls. Also Wagner 1 and 2 graded patients had higher serum zinc levels compared to healthy controls ($p < 0.001$). This study confirms that trace elements may be used as a leading sign to determine the severity of infections in diabetic foot wounds and may be used to predict outcome of diabetic foot infections.

Key words: Copper, zinc, diabetic foot and classification.

INTRODUCTION

Diabetic foot infection (DFI) is a serious complication of diabetes mellitus. Approximately 10 to 25% of all diabetics develop some foot problems during the course of their illness from simple calluses to major abscesses and osteomyelitis (Rooh et al., 2003). In the literature, several classification systems for diabetic foot ulcers have been proposed. The most widely used and validated system is the Meggitt Wagner classification (Boulton et al., 2000).

It was postulated that certain metals or metalloids might have specific roles in the pathogenesis and progress of diabetes mellitus (Zheng et al., 2008; Meyer and Spence,

2009; Valko et al., 2005; Kornhauser et al., 2008) and trace element status in diabetic patients is altered as compared to healthy subjects (Aguilar et al., 2007). The deficiency of essential elements may lead to the failure of antioxidant defense and also to glucose intolerance, both important in the progress of diabetes and its complications. Furthermore, the overload of some transition metals may be responsible for oxidative damage (Valko et al., 2005).

This prospective study was performed to evaluate the effect of trace elements in diabetic foot disease presenting with different severity of infections.

MATERIALS AND METHODS

This prospective study was carried out in 50 diabetic patients complicated with infections DFI. All patients were hospitalized in the

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Table 1. Wagner's classification for diabetic foot disease.

Grade	Characteristics
Grade 0	High risk foot and no ulceration.
Grade 1	Superficial ulcer, skin involvement
Grade 2	Deep ulcer (Skin and soft tissue involvement)
Grade 3	Osteomyelitis (Skin, soft tissue and bone involvement)
Grade 4	Gangrenous patches.
Grade 5	Gangrene of entire foot.

clinical services of the Clinical Microbiology and Infectious Diseases, Orthopedics and Endocrinology Clinics at Dicle University Hospital, between October 2006 and November 2008. As a control group; 50 age and sex matched healthy subjects were taken to the study. All groups had a detailed history taken and underwent a through clinical examination. Fasting serum glucose, HbA1C, complete blood examination, complete urine examination, routine biochemical tests and Xray foot graphics were measured from all groups. Deep tissue cultures were performed from all diabetic foot infected patients. The wound or ulcer on the foot was carefully evaluated and graded according to Wagner's classification (Table 1), considering the severity of infection at the time of presentation. Other parameters may effect trace element levels and diabetic foot so logistic regression analysis was performed using each parameter (age, blood glucose level, BUN, creatinin and HbA1c.

The trace elements were measured in all serum samples by using Unicam 929 Atomic Absorption Spectrophotometer. From all groups (n = 100), 5 ml venous blood was taken from the antecubital vein after overnight fasting. The blood was centrifuged at 5200 rpm for 10 min. Then blood samples were stored in deepfreezer, at 80°C. At the time of study, serum samples were diluted with deionized water. Copper (Cu), zinc (Zn) and Mg levels were measured in all serum samples by using Unicam 929 Atomic Absorption Spectrophotometer. Informed consent was obtained from all patients who participated to our study. This study was approved by the Ethics Committee of the Dicle University. The study protocol conforms to the ethical guidelines of the 1975 declaration of Helsinki.

Statistics

All data were statistically summarized with the mean and standard deviation (SD). Independent variables, we wanted to determine whether the effects of dependent variables. Trace element variables, according to disease groups, showing change over and examined the phases of disease. To examine the of different factor groups; The Tukey B test for multiple comperations was used. General Linear Model (GLM) Univariate procedure was used with SPSS software. Tukey B range test was used in the serum trace elements comparisons. Statistical analyses were performed by using SPSS 17.0 for Windows Version program. P < 0.05 values were considered statistically significant. Logistic regression analysis was used using each datas.

RESULTS

Of 25 DFI patients with Wagner stages 1 and 2; 13 (52%) were male and 12 (48%) were female (mean age = 57.6 ± 10.3 years). Of 25 DFI patients with Wagner stages 3 and 4; 13 (52%) were male and 12 (48%) were female

(mean age = 52.1± 12.8 years). Of 50 control healthy subjects; 25 (50%) were male and 25 (50%) were female (mean age = 48.5 ± 6.1 years).

In comparison of trace element levels; serum Cu levels were significantly higher in DFI patients with Wagner 3 and 4 as compared to DFI patients with Wagner 1 and 2 (p < 0.003) and healthy controls (p < 0.001). In comparison of serum Zn levels; serum Zn levels were significantly higher in all DFI patients with Wagner stages 1, 2, 3 and 4 than healthy controls (p < 0.001). Also, in severe infected patients, Wagner stages 3 and 4, serum Zn level was higher when compared to milder infected patients, Wagner stages 1 and 2 (p < 0.001).

However, serum Mg levels were significantly lower in Wagner 3 and 4 graded DFI group versus Wagner 1 and 2 graded DFI and healthy groups (p < 0.001). The ratio of Cu/Zn levels were statistically lower in Wagner 3 and 4 compared to healthy controls (p < 0.001). There was a tendency toward higher concentrations of Cu and Zn in Wagner 3 and 4 DFI patients versus healthy and Wagner 1 and 2 graded DFI and hypomagnesaemia in severe infected patients than healthy controls. The comparison of serum trace element levels in Wagner stages 1 and 2 with Wagner stages 3 and 4 were shown in Table 2.

The comparison of serum trace element levels in Wagner 1 and 2 versus healthy controls were shown in Table 3. The comparison of serum trace element levels in Wagner 3, 4 and healthy controls were shown in Table 4. HbA1c (p = 0.013), blood glucose level (p = 0.004), age (P = 0.031) was found as significant variables in wagner 3 and 4 regarding wagner 1 and 2. The constant number was decreased in wagner 3 and 4 (Exp(b) = 0.003) regarding wagner 1 and 2.

DISCUSSION

Diabetes mellitus-related foot infection is very common. There are many studies evaluating the role of trace elements in diabetic patients but in our knowledge, this study is the first study comparing trace element levels in diabetic foot infected patients classified as Wagner stages 1, 2, 3 and 4.

Trace elements, especially Zn and Cu play an important role in organism and host defense. Zinc and Copper metabolism of the host is markedly altered in all

Table 2. The comparison of Wagner 1, 2 and Wagner 3, 4.

Trace elements	Wagner 1+2	Wagner 3+4	P. value
Cu ($\mu\text{g/dL}$) \pm Sd*	63.8 \pm 17.4	81.8 \pm 21.2	0.003
Zn ($\mu\text{g/dL}$) \pm Sd	76.6 \pm 15.1	97.4 \pm 27.9	0.0001
Mg ($\mu\text{g/dL}$) \pm Sd	1160 \pm 328	1130 \pm 190	0.66
Cu/Zn ($\mu\text{g/dL}$) \pm Sd	0.85 \pm 0.2	0.90 \pm 0.3	0.65

Table 3. The comparison of Wagner 1, 2 and control groups.

Trace Elements	Wagner 1+2	Control	P value
Cu ($\mu\text{g/dL}$) \pm Sd*	63.8 \pm 17.4	58.8 \pm 19.2	0.52
Zn ($\mu\text{g/dL}$) \pm Sd	76.6 \pm 15.1	54.2 \pm 11.4	0.0001
Mg ($\mu\text{g/dL}$) \pm Sd	1160 \pm 328	1435 \pm 297	0.0001
Cu/Zn ($\mu\text{g/dL}$) \pm Sd	0.85 \pm 0.2	1.11 \pm 0.3	0.003

Table 4. The comparison of Wagner 3, 4 and control groups.

Trace elements	Wagner 3+4	Control	P value
Cu ($\mu\text{g/dL}$) \pm Sd*	81.8 \pm 21.2	58.8 \pm 19.2	0.0001
Zn ($\mu\text{g/dL}$) \pm Sd	97.4 \pm 27.9	54.2 \pm 11.4	0.0001
Mg ($\mu\text{g/dL}$) \pm Sd	1130 \pm 190	1435 \pm 297	0.0001
Cu/Zn ($\mu\text{g/dL}$) \pm Sd	0.90 \pm 0.3	1.11 \pm 0.3	0.01

Table 5. The comparison of Wagner 3, 4 and Wagner 1, 2 groups using logistic regression analysis.

Observed	Predicted		Percentage (%)
	Wagner group		
	Wagner 3-4	Wagner 1-2	
Wagner 3-4	19	6	76.0
Wagner 1-2	7	18	72.0
Overall percentage			74.0

infections or inflammations (Kalkan et al, 2000). Our data confirmed that trace elements were more altered in severe inflammations compared to milder infections. Serum zn and cu levels were more significantly increased in diabetic foot infected patients classified as Wagner 3 and 4 compared to Wagner 1 and 2 graded patients and healthy controls. However, Serum Zn and Cu and Mg levels were significantly decreased in Wagner 3 and 4.

Trace elements, which are co-factors of enzymes in antioxidant defense system, are often investigated in many diseases, such as DM and similar pathogenic diseases. In these studies, contradictory results are reported in terms of plasma or serum levels of trace elements. As regards copper, there are studies reported in serum and plasma levels as higher (Raz and Havivi, 1989), lower (Car et al., 1992) and normal (Mooradian

and Morley, 1987) and is being compared with control groups. In our study, serum Cu levels were found at its highest levels in Wagner 3 and 4 versus Wagner 1 and 2.

The studies suggesting that abnormal Zn metabolism may play a predictive role in the pathogenesis of DM and development of its complications, bringing Zn related topics current (Kinlaw et al., 1983). There are also several studies reporting Zn levels in diabetic patients as low (Valter et al., 1991) or high (Abdul et al., 2002). The findings of our study clearly show that serum zinc levels in severe infected patients, Wagner stages 3 and 4, were significantly higher with respect to milder infections, Wagner stages 1 and 2.

In many studies, it was reported that chronic infections and inflammations affect body metabolism and cause alterations at Cu and Zn levels (Sonmez et al., 1997;

Table 6. Variables (HbA1c, blood glucose level, Age, BUN, creatinin) were shown in the equation using logistic regression analysis.

Variables	B	S.E	Wald	df	Sig.	Exp (B)	95% C.I. for EXP(B)	
							Lower	Upper
HbA1c	-583	235	6.169	1	013	558	353	884
Glucose	041	014	8.422	1	004	1.041	1.013	1.070
BUN	010	015	453	1	501	1.010	980	1.042
Creatinin	1.182	1.339	780	1	377	3.262	237	44.976
Age	071	033	4.632	1	031	1.073	1.006	1.145
Constant	-5.858	2.453	5.702	1	017	0.003		

Table 7. Variables (HbA1c, blood glucose level, Age, BUN, creatinin) were compared using logistic regression analysis .

Variables		N	Mean	Std. Deviation	95% Confidence interval for mean	
					Lower bound	Upper bound
Age F=8.15 P=0.001	Wagner 1-2	25	57.68	10.375	53.40	61.96
	Wagner 3-4	25	52.12	12.853	46.81	57.43
	Controls	50	48.50	6.135	46.76	50.24
	Total	100	51.70	9.951	49.73	53.67
HbA1c F=83.58 P=0.000	Wagner 1-2	25	9.70	2.927	8.50	10.91
	Wagner 3-4	25	9.43	2.476	8.41	10.45
	Controls	50	4.47	0.711	4.27	4.67
	Total	100	7.02	3.223	6.38	7.66
Fasting blood glucose F=75.95 P=0.001	Wagner 1-2	25	166.52	39.966	100.02	183.02
	Wagner 3-4	25	138.56	40.021	122.04	155.08
	Controls	50	82.74	12.152	79.29	86.19
	Total	100	117.64	46.665	108.38	126.90
Urea F=1.58 P=0.211	Wagner 1-2	25	47.32	31.736	34.22	60.42
	Wagner 3-4	25	35.84	33.161	22.15	49.53
	Controls	50	38.78	11.086	35.63	41.93
	Total	100	40.18	24.294	35.36	45.00
Creatine F=11.95 P=0.001	Wagner 1-2	25	0.88	0.362	0.73	1.03
	Wagner 3-4	25	0.77	0.355	0.63	0.92
	Controls	50	0.55	0.211	0.49	0.61
	Total	100	0.69	0.325	0.63	0.75

Yuregir et al., 1997). This elevation might be due to release of copper from intracellular part of the cell to the extracellular environment because of bacterial invasion (Sonmez et al., 1997), through releasing some antioxidants by neutrophils may cause an increased in the levels of Zn and Cu as a consequence of cellular damage (Fliss and Menard, 1992). Moreover, it was thought that elevated levels of minor acute phase reactants, such as ceruloplasmin, could be a reason for the increased serum Cu levels in infections (Benzer and Kilic, 2006).

In the studies carried out in diabetic patients, generally hypomagnesaemia is reported by Abdul et al. (2002). In

our study, serum Mg levels in severe DFI cases were found to be significantly lower with respect to milder DFI patients. In DM, although the cause of hypomagnesaemia has not been exactly understood, Mg is blamed to impair the absorption of Mg from renal tubular system.

Conclusion

Our study was of a different nature due to the fact that it was the first study carried out on trace element changes

in Wagner classified diabetic foot infected patients. The other difference from other studies is that we found serum zinc levels significantly increased in diabetic foot infected patients especially in severe cases, classified as Wagner 3 and 4, unlike other studies conducted in diabetic patients and other diseases inducing oxidative stress.

The present study shows that trace elements are altered in diabetic foot infected patients and provides confirmatory results, indicating that trace elements in diabetic foot infected patients is disturbed in wagner 3 and 4 as compared to milder infections in wagner 1 and 2. We conclude that trace elements may be used as a leading sign to determine the severity of infections in diabetic foot wounds and to predict outcome of diabetic foot infections.

In conclusion, more extensive studies should be carried out in terms of serum trace elements in chronic and severe cases such as DFI patients in order to understand their role better in the pathogenesis, diagnosis and treatment.

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