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Original article

Changes in urinary Cu, Zn, and Se levels in cancer patients after treatment with Sha Shen Mai Men Dong Tang

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ABSTRACT

Sha Shen Mai Men Dong Tang (SMD-2; 沙參麥冬湯 shā shēn mài dōng tāng) is a Chinese medicinal herb (CMH; 中草藥 zhōng cǎo yào) used to treat symptoms associated with cancer therapy. The objective of this study was to assess the effect of SMD-2 on the levels of urinary copper (Cu), zinc (Zn), and selenium (Se) in lung cancer patients and head and neck cancer patients receiving chemoradiotherapy. Forty-two head and neck cancer patients and 10 lung cancer patients participated in our clinical trial. Each patient received chemoradiotherapy for 4 weeks. In addition, each patient was treated with SMD-2 for 8 weeks, including 2 weeks prior to and after the chemoradiotherapy treatment. Comparison of urinary Cu, Zn, and Se levels and the ratios of Zn to Cu and Se to Cu at three time points in the two types of cancer were assessed using the generalized estimating equations (GEEs). After the patients received chemoradiotherapy for 4 weeks, SMD-2 treatment was found to be associated with a significant decrease in urinary Cu levels, whereas urinary Zn and Se levels increased significantly. In addition, the ratios of Zn to Cu and Se to Cu in the urine samples of these patients also increased significantly. Both the urinary Zn levels and the ratio of Zn to Cu in head and neck cancer patients were significantly higher than in lung cancer patients. Urinary Zn and Se levels and the ratios of Zn to Cu and Se to Cu, but not urinary Cu levels, increased significantly during and after treatment when assessed using the GEE model. The SMD-2 treatments significantly increased Zn and Se levels in the urine of head and neck cancer patients. Increased Zn and Se levels in urine strengthened immune system.

1. Introduction

In Taiwan, the practice of using Chinese medicine in combination with Western medicine to treat cancer patients is on the rise. In this study, we treated head and neck as well as lung cancer patients undergoing radiotherapy or chemotherapy with Sha Shen Mai Men Dong Tang (SMD-2; 沙參麥冬湯 shā shēn mài dōng tāng), a mixture of seven herbs. According to Chinese medicine, SMD-2 can "clear and nourish the lungs and stomach (清肺養胃 qīng fèi yǎng wèi)", "engender fluid (生津 shēng jīn)", repair damaged cells, and control the symptoms of dryness associated with cancer treatment. Western cancer treatment depends solely on the use of radiotherapy or chemotherapy, which unfortunately produces serious side effects. Short-term side effects of chemoradiotherapy include fatigue, nausea, vomiting, mucositis, myelosuppression, and neutropenia. These side effects generally occur within months of completion of chemotherapy.[1] A variety of Chinese medicinal herbs (CMHs; 中草藥 zhōng cǎo yào) have been used to minimize these side effects. A clinical study performed by Lan[2] in China indicated a significant reduction in oral mucosal symptoms in nasopharyngeal cancer patients who first received chemoradiotherapy and then SMD-2 treatments. Another study in China by Sung and Dong[3] showed that head and neck cancer patients who received

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chemoradiotherapy experienced oral dryness and had difficulty chewing and swallowing. The patients were then treated with SMD-2, and their symptoms improved tremendously.

Previous studies have shown that fluctuations in the levels of Se, Zn, and Cu in humans are indicators of cancer.[4,5] For example, high serum Cu levels and low serum Zn levels are markers of lung cancer.[6] Songchitsomboon et al[7] suggested that the use of serum Cu to Zn ratios as markers for the diagnosis of cancer or for staging tumors must be interpreted with caution. A double-blind, randomized study conducted by Lin et al[8] indicated that after head and neck cancer patients received chemoradiotherapy, Zn supplements repair cellular DNA, strengthen the immune system, and alleviate the severity of mucositis and dermatitis. As mentioned earlier, the levels of Zn, Se, and Cu fluctuate in cancer patients; therefore, monitoring the levels of these trace elements during cancer treatment may help in understanding the efficacy of cancer treatment, as well as provide an important tool in the diagnosis of cancer. The objective of this study was to assess the effect of SMD-2 on changes in urinary Cu, Zn, and Se levels in lung cancer patients and head and neck cancer patients receiving chemoradiotherapy.

2. Materials and methods

2.1. Study design and participant selection

Measurements of urinary Cu, Zn, and Se levels were obtained prior to and after SMD-2 treatment. This protocol was approved by the Institutional Review Board in China Medical University, and informed consent was obtained from patients prior to their participation. Ten lung cancer patients and 42 head and neck cancer patients participated in our study, which was conducted at a medical center in central Taiwan. Each patient was advised by medical personnel to take vitamins containing Zn and Se. Because of ethical considerations, a placebo group was not included in the study design. Each cancer patient was treated with radiotherapy (average dose, 5440 cGy) and chemotherapy (cisplatin, 60 mg/m^2 on Day 1; 5-fluorouracil, 500 mg/m^2/day on Days 1–5; and mitomycin C, 6 mg/m^2/day on Day 1 and Day 8) for 4 weeks. In addition, each patient received SMD-2 treatments for 8 weeks (2 weeks prior to receiving chemoradiotherapy, 4 weeks during chemoradiotherapy, and 2 weeks after the treatment). SMD-2 is a powder made of Adenophorae radix (南沙參 nán shā shēn), *Ophiopogon* tuber (麥門冬 mài mén dōng), *Polygonatum officinale* rhizome (玉竹 yù zhú), folium mori (桑葉 sāng yè), *Trichosanthes* root (天花粉 tiān huā fěn), semen dolichoris (白扁豆bái biǎn dòu), *Polygonatum odoratum* (葳蕤 wēi ruí), and *Glycyrrhiza* radix (甘草 gān cǎo). SMD-2 is prepared by a manufacturer in Taiwan with good manufacturing practices. We characterized SMD-2 with inductively coupled plasma/mass spectrometry (ICP/MS) and found it to contain Cu (27.6 ppm), Zn (85.7 ppm), and Se (0.38 ppm).

2.2. Measurement

Fresh spot urine samples were obtained from each cancer patient at three points during the trial (prior to, during, and after chemoradiotherapy). Specimens were stored at −70°C until metal analysis was carried out. An ICP/MS instrument (PerkinElmer ELAN DRC-e, Waltham, Massachusetts, US) was used to measure the Cu, Zn, and Se levels. Jones[9] and Heitland and Köster[10] have described this procedure in detail. The methodology for metal analysis and quality control has been published previously. The instrument detection limit for Cu, Zn, and Se using ICP/MS was 0.16 μg/L, 0.48 μg/L, and 0.29 μg/L, respectively. The method detection limits for Cu, Zn, and Se were 0.17 μg/L, 2.53 μg/L, and 0.30 μg/L, respectively. The precision (relative standard deviation) of measuring urinary metals was less than 5%. The recovery for measuring urinary metals was 106% for Cu, 102% for Zn, and 104% for Se.

2.3. Data collection and analysis

After measuring urinary metal levels, we calculated the ratios of Zn to Cu (Zn/Cu ratio) and Se to Cu (Se/Cu ratio). Data were collected using Microsoft Excel 2000 (Microsoft, Redmond, WA, USA) and analyzed by SPSS version 17.0 (SPSS, Inc., Chicago, IL, USA). Because the sample size in this study was limited, the nonparametric method was used. The differences in urinary metal levels (μg/L) for each intervention period were examined using the Mann–Whitney U test ($p < 0.05$). The urinary Cu, Zn, and Se levels and the ratios of Zn/Cu and Se/Cu in the two types of cancer at three-stage points were compared using the generalized estimating equations (GEEs).

3. Results

Table 1 presents the urinary Cu, Zn, and Se levels prior to, during, and after the treatment in patients with cancer of the head and neck who were treated using SMD-2. The level of Cu in the urine of these patients decreased to 25 μg/L after the treatment; however, the urinary Zn and Se levels increased to 578 μg/L and 78 μg/L, respectively, after the treatment. Furthermore, the Zn/Cu and Se/Cu ratios in the urine of these patients increased 111 times and 18 times, respectively.

Table 2 presents the urinary Cu, Zn, and Se levels prior to, during, and after the treatment in patients with lung cancer who were treated using SMD-2. Prior to treatment, the urinary Cu level in these patients was 25.9 μg/L on average, but after the treatment, the average urinary Cu level decreased to 8.55 μg/L, which was statistically significant. Furthermore, the urinary Zn levels increased by more than threefold after the treatment, and urinary Se levels doubled after the treatment, both of which were also statistically significant. In addition, the Zn/Cu and Se/Cu ratios in the urine of these patients increased significantly.

Table 3 presents a comparison of the urinary Cu, Zn, and Se levels and the ratios of Zn/Cu and Se/Cu in the two types of cancer at three time points using the GEE model. There was no significant difference in urinary Cu levels in patients suffering from the two types of cancer. Both urinary Zn levels and the ratio of Zn/Cu in patients with head and neck cancer were significantly higher than in those with lung cancer. However, there was no significant difference in the urinary Cu and Se levels (μg/L), or in the ratio of Se/Cu between the groups of cancer patients. In addition, urinary Zn and Se levels (μg/L) and the ratios of Zn/Cu and Se/Cu, but not urinary Cu levels, increased significantly during and after the treatment.

Table 1
Urinary Cu, Zn, and Se levels (μg/L) and ratios of Zn/Cu and Se/Cu prior to, during, and after the SMD-2 treatment in patients with cancer of the head and neck ($N = 42$).

	Prior to treatment	During treatment	After treatment	p_1*	p_2**
Cu	33.95 ± 52.00	18.82 ± 20.47	8.33 ± 6.81	<0.001	<0.001
Zn	212.2 ± 274.1	491.79 ± 718.8	790.8 ± 863.0	<0.001	<0.001
Se	37.44 ± 35.74	67.33 ± 59.45	115.83 ± 862.99	<0.001	<0.001
Zn/Cu	11.01 ± 15.30	32.72 ± 39.27	122.14 ± 114.37	<0.001	<0.001
Se/Cu	2.08 ± 2.20	4.84 ± 301	20.80 ± 16.46	<0.001	<0.001

*p_1 was calculated using paired t test between the periods prior to and during treatment.
**p_2 was calculated using paired t test between the periods prior to and after treatment.
SMD-2 = Sha Shen Mai Men Dong Tang.

Table 2
Urinary Cu, Zn, and Se levels (μg/L) and ratios of Zn/Cu and Se/Cu prior to, during, and after the SMD-2 treatment in patients with lung cancer ($N = 10$).

	Prior to treatment	During treatment	After treatment	p_1*	p_2**
Cu	25.93 ± 30.64	18.78 ± 21.36	8.55 ± 7.44	0.008	0.003
Zn	50.90 ± 38.23	66.94 ± 77.60	164.73 ± 96.65	0.013	0.003
Se	29.45 ± 24.58	41.42 ± 26.98	69.75 ± 31.88	0.021	0.003
Zn/Cu	4.89 ± 7.90	8.01 ± 9.43	30.82 ± 21.78	0.003	0.003
Se/Cu	1.80 ± 1.20	4.14 ± 3.56	13.97 ± 8.99	0.003	0.003

*p_1 was calculated using paired t test between the periods prior to and during treatment.
**p_2 was calculated using paired t test between the periods prior to and after treatment.
SMD-2 = Sha Shen Mai Men Dong Tang.

4. Discussion

Our study showed that, in cancer patients who received 8 weeks of SMD-2 treatments and 4 weeks of chemoradiotherapy, the urinary Zn and Se levels and the ratios of Zn/Cu and Se/Cu increased significantly. Conversely, urinary Cu levels decreased significantly during and after treatment. SMD-2 contains the following eight ingredients: hyacinth bean (白扁豆bái biǎn dòu), *Trichosanthes* root (天花粉 tiān huā fěn), *Polygonati officinalis rhizoma* (玉竹 yù zhú) *Polygonatum odoratum* (葳蕤 wēi ruí), Adenophorae radix (南沙參 nán shā shēn), folium mori (桑葉 sāng yè), *Ophiopogon* tuber (麥門冬 mài mén dōng), and *Glycyrrhiza* radix (甘草 gān cǎo)

Traditional indications for SMD-2 include a dry throat, thirst, and a hacking cough with little sputum. Cancer patients' lungs are often deficient in "Yin (陰 yīn)" which results in the aforementioned symptoms. *Glehnia* root is used to "clear the lungs and activate Yin (養陰清肺 yǎng yīn qīng fèi)" which "strengthens the stomach (健胃 jiàn wèi)" and "promotes the production of bodily fluids (生津 shēng jīn)". *Ophiopogon* root tuber is used to "nourish the Yin, preventing the lungs from becoming too dry (滋陰潤肺 zī yīn rùn fèi)", and "remove excess heat from the heart (清心熱 qīng xīn rè)", as well as to help people relax.

SMD-2 treatments are beneficial to cancer patients who receive radiotherapy because Zn promotes collagen synthesis and epithelialization, both of which are associated with the healing process. Zn-binding proteins can be sequestered by mucosal cells and transferred to albumin molecules on the serous site of the mucosal cell membrane. However, Cu can interfere with Zn absorption by competing for binding sites on the albumin molecule.[11,12] The chemopreventive properties of Se may be responsible for the anticancer effects of compounds containing this element.

Chemoradiotherapy damages cell membranes and harms the immune system in most cancer patients. In our study, every head and neck cancer patient who received chemoradiotherapy experienced side effects such as oral dryness and difficulty in chewing and swallowing. SMD-2 treatments significantly reduced these side effects, decreased the patients' pain, and increased the patients' energy levels and appetite (data not shown). According to Chinese medicine, an imbalance between "Yin and Yang (陰陽 yīn yáng)" will cause disease. SMD-2 was used to treat symptoms including damage to the lungs and stomach, "fluid-humor depletion (津液不足 jīn yè bù zú)", "dry throat and thirst (口乾作渴 kǒu gān zuò kě)", and "dry cough with low phlegm production (乾咳少痰 gān ké shǎo tán)". Seki et al[13] showed that patients who underwent radiotherapy exhibited "fire due to Yin deficiency (陰虛火旺 yīn xū huǒ wàng)". After receiving chemotherapy, patients exhibited a "deficiency of Qi and Yin (陰氣兩虛 qì yīn liǎng xū)". Patients who were terminally ill had a "deficiency of both Yin and Yang (陰陽兩虛 yīn yáng liǎng xū)". Radiotherapy produces excessive heat "Yin (陰 yīn)" in cancer patients, and this heat cannot be released in a timely manner. When cancer patients undergoing chemoradiotherapy were evaluated using the diagnostic methods of traditional Chinese medicine (中醫 zhōng yī; TCM), typical symptoms included "blood deficiency (血虛 xuè xū)", "splenic asthenia (脾虛 pí xū)", and "stagnation of liver Qi (肝鬱氣滯 gān yù qì zhì)". Therefore, there are imbalances in Qi and blood, which result in severe damage to the liver and kidney.

Most TCM remedies are mixtures of diverse herbs. The goal of "reverse phytochemistry" is to isolate active compounds from different herb mixtures and combine them to create novel composite products.[14] According to Chinese documents, SMD-2 is beneficial for "activating blood (活血 huó xuè)", "dissolving stasis (化瘀 huà yū)", "clearing heat (清熱 qīng rè)", and "improving expectoration (化痰止咳 huà tán zhǐ ké)". In addition, SMD-2 "clears Ying heat (清陰熱 qīng yīn rè)" and "cools the blood (涼血 liáng xuè)" to improve removal of "Ying heat (陰熱 yīn rè)" from cancer patients. The traditional use of SMD-2 is to "nourish the blood and soothe the liver (疏肝養血 shū gān yǎng xuè)". SMD-2 not only acts as the prescription but also reflects therapeutic principles. Overall, the combined impact of SMD-2 on the three common symptoms of cancer is to "nourish the blood (養血 yǎng xuè)", and thus "invigorate the spleen and soothe the liver (健脾疏肝 jiàn pí shū gān)" to "disperse stagnation (散瘀 sàn yū)".

Head and neck cancer is the most common cancer in Taiwan. Overall, the survival rate among patients with advanced head and neck cancer is often poor, because of significant medical comorbidities and the development of second primary cancers, which have been associated with both betel nut and tobacco overuse.[15] Although the combination of chemotherapy and radiation therapy has the advantage of different treatment modalities with different toxicity profiles, chemoradiotherapy to treat cancer in the head and neck is an aggressive method associated with major toxicity. Patients who receive this treatment often develop various

Table 3
Comparison of the urinary Cu, Zn, and Se levels (μg/L) and ratios of Zn/Cu and Se/Cu in the two types of cancer at the three time points using the generalized estimating equation model.

	Cu β (SE)	Zn β (SE)	Se β (SE)	Zn/Cu β (SE)	Se/Cu β (SE)
Cancer types					
Head and neck	3.03 (8.28)	393.9 (183.1)*	29.0 (17.2)	39.0 (16.3)*	2.20 (2.07)
Lung	Reference	Reference	Reference	Reference	Reference
Stages					
Prior to treatment	23.6 (4.7)**	−480.2 (72.3)**	−70.4 (7.54)**	−93.2 (10.9)**	−17.3 (1.67)**
During treatment	10.2 (4.7)**	−252.4 (72.3)**	−44.1 (7.54)**	−75.3 (10.9)**	−14.6 (1.67)**
After treatment	Reference	Reference	Reference	Reference	Reference

*$p < 0.05$.
**$p < 0.01$.
SE = standard error.

side effects, such as mucositis and dermatitis.[16] Almost a third of UK cancer patients sampled in a previous study used some form of complementary and alternative medicine (CAM; 補充與替代醫學 bǔ chōng yǔ tì dài yī xué), with the most common therapies being relaxation/meditation techniques, spiritual or faith healing, medicinal teas, reflexology, aromatherapy, herbal therapy, and vitamin or mineral therapy.[17] CMHs are sometimes used as an adjunct to radiotherapy or chemotherapy for cancer. Evidence from current studies is weakened by methodological limitations. Because of conflicting reports, it is difficult to argue for or against the use of CMH as a treatment for cancer.[1,18] The majority of studies have shown that CMH improved treatment side effects and performance status, and some have provided evidence of tumor regression and increased survival.[19] Seven randomized controlled trials have been conducted comparing chemotherapy with or without Chinese herbs in 542 female breast cancer patients in China. Three trials indicated an improvement in white blood cells in the group receiving CMH. Two showed an increase in percentage changes in T-lymphocyte subsets CD4 and CD8. One study showed a statistically significant difference in percentage changes in T-lymphocyte subsets CD3, CD4, and CD8 associated with TCM.[1] Although these studies did not investigate specific components of CMH, these supplements may have a role in stimulating immunological function in cancer patients. Consistent with this concept, SMD-2 can be used to modify imbalances in Qi and blood and repair cell damage to ensure maintenance of optimal immunity. Our patients using SMD-2 treatment had increases in body weight and reported improvements in quality of life with regard to sleep deprivation and anorexia (data not shown). Therefore, side effects or symptoms in cancer patients using chemoradiotherapy can be alleviated, reinforcing normal diet intake and mediating their body functions. Seki et al[13] hypothesized that if cancer patients for whom "Yin and Yang are deficient (陰陽兩虛 yīn yáng liǎng xū)" were treated with "Yang and Qi tonics (補陽益氣 bǔ yáng yì qì)" during the day, sympathetic activation of natural killer cells would increase, thus improving defense against malignant cells.[13]

In this study, trace elements of SMD-2 were analyzed using ICP/MS. We found that magnesium (Mg; 47,440 μg/g) was present at the highest levels, followed by potassium (K; 25,265 μg/g) and calcium (Ca; 12,591 μg/g). The level of Zn present was 85.7 μg/g. The levels of Cu and Se were considerably limited. In our SMD-2 treatment study, we did not find a great increase in levels of trace elements in urine. However, a threefold increase in the urinary Zn levels and a 10-fold increase in the Zn/Cu ratio were detected after head and neck cancer patients were treated with chemoradiotherapy. In this study, Zn also had an effect in improving the healing process for patients with mucositis and dermatitis caused by radiation. It has been previously shown that Zn plays a significant role in the context of metabolic response to injury and wound healing,[20] but its role in the healing of radiation-related mucositis and dermatitis is not entirely clear. However, Zn is known to be required by > 80 different enzymes including alcohol dehydrogenase, carbonic anhydrase, DNA and RNA polymerase, and carboxypeptidase. Consequently, when elevated levels of Zn are present, tumors may continue to grow despite the lack of new vessel formation, which may result in necrosis. These findings could explain the presence of elevated Zn levels in secondary liver metastases compared with primary colorectal tissues.[21] Moreover, a recent study by Rudolf et al[22] has shown that Zn has the potential to be used in chemoprevention of advanced colorectal carcinoma. Zn may help suppress free radicals through metallothionein synthesis, act as an inhibitor of the nicotinamide adenine dinucleotide phosphate (reduced)-dependent lipid peroxidation, and prevent lipid peroxidation by inhibition of glutathione depletion.[23] However, Cu can interfere with Zn absorption by competing for binding sites on the albumin molecule in the intravascular space.[12] Interestingly, our findings also indicated a statistically significant fourfold decrease in urinary Cu levels after cancer patients were treated with chemoradiotherapy. The absorption of Cu into the human body is a complicated process, depending on various factors and dietary components. Excess Cu has been known to be a potent oxidant, causing the generation of radical oxygen species in cells. In addition, a study in Taiwan by Kuo et al[24] showed that Cu levels in serum and tumor cells have been reported to be significantly elevated in cancer patients compared with healthy individuals.

There are some limitations to this study. First, this was not a randomized clinical experiment. Because of ethical considerations, no placebo group was included in the study. Eighty percent of the participants were head and neck cancer patients. All cancer patients were monitored for urinary metal levels at three time points during the SMD-2 treatment period. The difference between urinary metal levels was measured in two cancer patient groups undergoing SMD-2 treatment to account for the two types of cancer and individual variability. Urinary creatinine was not measured in this study. Because urinary metal levels were measured at three points over 8 weeks, we assumed a stable urinary creatinine level in each cancer patient. Second, no accurate measure of intake of trace elements from other sources was recorded during cancer treatment. Each cancer patient may alleviate the side effects from chemoradiotherapy using SMD-2, as well as Zn and Se from various other sources. These trace elements can repair damaged cells and boost immune function. However, we observed that urinary Zn and Se levels in cancer patients using SMD-2 significantly increased. In addition, due to the small sample size and limited follow-up time, further research is necessary to elucidate the active ingredients of SMD-2 and their pharmacological activities, toxicity, and clinical efficacy.

5. Conclusion

SMD-2 treatments significantly increased Zn and Se levels in the urine samples from head and neck cancer patients, which will strengthen their immune system. We conclude that increased Zn and Se may account for the anticancer activity of SMD-2, which has been used for a number of years in traditional medicine to promote resistance to cancer. These findings may provide a basis for the isolation and identification of more highly effective anticancer components.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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