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

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A B S T R A C T

Sha Shen Mai Men Dong Tang (SMD-2; 沙參麥冬湯 sha shen mai dòng tang) is a Chinese medicinal herb (CMH; 中草藥 zhòng 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.

Keywords:
chemoradiotherapy
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ratio of Zn to Cu

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 sha shen mai dòng tang), a mixture
of seven herbs. According to Chinese medicine, SMD-2 can “clear
and nourish the lungs and stomach (清肺養胃 qīng fēi 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. A variety of Chinese medicinal herbs (CMHs; 中草藥 zhòng cǎo yào) have been used to minimize these
side effects. A clinical study performed by Lan2 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 Dong3
showed that head and neck cancer patients who received
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. For example, high serum Cu levels and low serum Zn levels are markers of lung cancer. Songchitsomboon et al. 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. 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/m²) as part of the study design. Each cancer patient was treated with radiotherapy and chemotherapy (cisplatin, 60 mg/m²/day on Days 1–5; fluorouracil, 500 mg/m²/day on Days 1–5; and mitomycin C, 6 mg/m²/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 nà shā cān), Ophiopogon tuber (麥冬 mài dōng), Polygonatum officinale rhizome (玉竹 yù zhú), folium mori (桑葉 sāng yè), Trichosanthes root (天花粉 tiān huā fěn), semen dolichoris (白朮 bái shòu), 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' and Heitland and Köster’ 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 SMD-2 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

|                      | Prior to treatment | During treatment | After treatment | p1* | p2** |
|----------------------|-------------------|-----------------|----------------|-----|------|
| Cu                   | 33.95 ± 52.00     | 18.82 ± 20.47   | 8.33 ± 6.81    | <0.001 | <0.001 |
| Zn                   | 212.2 ± 274.1     | 481.79 ± 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 ± 1.20       | 4.84 ± 3.01     | 20.80 ± 16.46  | <0.001 | <0.001 |

*p1 was calculated using paired t test between the periods prior to and during treatment.

**p2 was calculated using paired t test between the periods prior to and after treatment.

SMD-2 — Sha Shen Mai Men Dong Tang.
immune system in most cancer patients. In our study, every head
treatment.

\[ \text{SE} \]

and after the SMD-2 treatment in patients with lung cancer (\( N = 30 \)).

| Prior to SMD-2 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 = She 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 blanco dou), Trichosanthes root (天花粉 tian hua fen), Polygonatum officinalis rhizoma (玉竹 yu zhu) Polygonatum odoratum (葳蕤 wei rui), Adenophorae radix (南沙参 nashacshen), folium mori (桑葉 sang ye), Ophiopogon tuber (麥門冬 mai mén dong), and Glycyrrhiza radix (甘草 gan cao).

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)” which results in the aforementioned symptoms. Glehnia root is used to “clear the lungs and activate Yin (養陰清肺 yang yin qing fei)” which “strengthens the stomach (健脾 jian wei)” and “promotes the production of bodily fluids (生津 sheng jin).” Ophiopogon root tuber is used to “nourish the Yin, preventing the lungs from becoming too dry (滋陰潤肺 zi yin run fei),” and “remove excess heat from the heart (清心熱 qing xin re),” 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 yin yang)” will cause disease. SMD-2 was used to treat symptoms including damage to the lungs and stomach, “fluid-humor depletion (津液不足 jin ye bu zu),” “dry throat and thirst (口乾作渴 kou gan zuo ke),” and “dry cough with low phlegm production (乾咳少痰 gan ke shao tan).” Seki et al13 showed that patients who underwent radiotherapy exhibited “fire due to Yin deficiency (陰虛火旺 yin xu huo wang).” After receiving chemotherapy, patients exhibited a “deficiency of Qi and Yin (陰氣不足 qin liang xu).” Patients who were terminally ill had a “deficiency of both Yin and Yang (陰陽兩虛 yin yang liang xu).” Radiotherapy produces excessive heat “Yin (陰)” 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 zhong yi; TCAM), typical symptoms included “blood deficiency (血虛 xue xu),” “splenic asthenia (脾虛 pi xu),” and “stagnation of liver Qi (肝鬱氣滞 gan yu qi zhi)).” Therefore, there are imbalances in Qi and blood, which result in severe damage to the liver and kidney.

Most TCAM 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 compound products.14 According to Chinese documents, SMD-2 is beneficial for “activating blood (活血 huoxue),” “dissolving stasis (化瘀 huiyu),” “clearing heat (清熱 qing re),” and “improving expectation (化瘀止血 huiyuzhihe).” In addition, SMD-2 “clears fire (清熱 qing re)” and “cools the blood (清涼 liang xue) to improve removal of “Ying heat (陰熱 yin re)” from cancer patients. The traditional use of SMD-2 is to “nourish the blood and soothe the liver (疏肝養血 shu gan yang xue).” 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 yang xue),” and thus “invigorate the spleen and soothe the liver (健脾疏肝 Jianpi shui gan)” to “disperse stagnation (散瘀 sanshu).”

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

| \( \text{Cu} \) (\( \mu \text{g}/\text{L} \)) | \( \text{Zn} \) (\( \mu \text{g}/\text{L} \)) | \( \text{Se} \) (\( \mu \text{g}/\text{L} \)) | \( \text{Zn/Cu} \) | \( \text{Se/Cu} \) |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Prior to treatment | 3.03 (8.28) | 393.9 (183.1)* | 29.0 (17.2) | 2.00 (2.07) |
| After treatment | 10.2 (4.7)** | 252.4 (72.3)** | 39.0 (16.3)* | 146 (1.6)** |

*p < 0.05.

**p < 0.01.

SE = standard error.

Table 2

| \( \text{Cu} \) (\( \mu \text{g}/\text{L} \)) | \( \text{Zn} \) (\( \mu \text{g}/\text{L} \)) | \( \text{Se} \) (\( \mu \text{g}/\text{L} \)) | \( \text{Zn/Cu} \) | \( \text{Se/Cu} \) |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Cancer types    | \( \text{T}-Y. \) Lai, H.-W. Kuo / Journal of Traditional and Complementary Medicine 6 (2016) 135–139 | 137 |
side effects, such as mucositis and dermatitis. 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) to ease discomfort. These common therapies include relaxation/meditation techniques, spiritual or faith healing, med- ical teas, reflexology, aromatherapy, herbal therapy, and vitamin or mineral therapy. 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. 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. 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 CMH. Although these studies did not investigate specific components of CMH, these supply disabled may have a role in stimulating immunological func- tion 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 improved- ments 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 allevi- ated, reinforcing normal diet intake and mediating their body functions. Seki et al hypothesized that if cancer patients for whom “Yin and Yang are deficient (陰陽兩虛, yín 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.

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 chemo- radiotherapy. 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, 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 dehydroge- nase, 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. Moreover, a recent study by Rudolf et al 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. However, Cu can interfere with Zn absorption by competing for binding sites on the albumin molecule in the intravascular space. Interestingly, our findings also indicated a statistically significant four-fold 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 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 pa- tients were monitored for urinary metal levels at three time points during the SMD-2 treatment period. The difference between urin- ary metal levels was measured in two cancer patient groups un- dergoing 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 treat- ment. Each cancer patient may alleviate the side effects from che- moradiotherapy 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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