0163-4984/02/8602–0137 $11.75

Determination of Some Elements in the Cervical Mucus of Healthy Taiwanese Women, by GF-AAS

I-Chuan Chuang,[1] Po-Ning Lee,[3] Te-Hsien Lin,[1] and Gwo-Shing Chen*,[2]

[1]Graduate Institute of Medicine and [2]Department of Dermatology, Kaohsiung Medical University, Kaohsiung, 807 Taiwan, Republic of China; and [3]Department of Obstetrics and Gynecology, Chi Mei Foundation Medical Center, Yung Kang City, Tainan, 710 Taiwan, Republic of China

Received May 31, 2001; Revised September 10, 2001;
Accepted September 19, 2001

ABSTRACT

The levels of seven trace elements such as copper, iron, zinc, selenium, cadmium, chromium, and nickel were determined by graphite furnace–atomic absorption spectrometry in the cervical mucus of 45 women residing in Taiwan, Republic of China. These women were in good health and had no precancerous or cancerous lesions on their cervix.

The women were separated into four age groups to establish if there was a relationship between the age of the subjects and the values of these elements in the cervical mucus. By one-way analysis of variance, significant differences in the selenium and nickel concentrations were found among the four age groups ($p < 0.05$). The mean concentration of selenium in each age group was significantly higher for the older women. A significant correlation between age and selenium concentration was found by linear regression ($r = 0.23$, $p = 0.00048$). No significant differences among the concentrations of copper, iron, zinc, cadmium, and chromium were found among these four age groups ($p > 0.05$).

The results presented in this work may be considered as baseline values for these elements in the cervical mucus of healthy Taiwanese women for use as reference in studies on cervical diseases and tissue damage.

Index Entries: Copper; iron; zinc; selenium; cadmium; chromium; nickel; cervical mucus; GF-AAS.

*Author to whom all correspondence and reprint requests should be addressed.

INTRODUCTION

The cervix plays an important role in the female reproductive system. Many gynecologic and obstetric clinical problems are caused by abnormal cervical conditions. Cervical mucus is secreted by the epithelial cells of the cervix and its main component is a high-molecular-weight colloid that consists of mucous glycoprotein. Because of normal hormonal and physical changes, the chemical components of the cervical mucus show cyclic changes over time *(1).*

Copper, iron, zinc, chromium, and selenium are recognized as essential trace elements, nickel is considered a nonessential element, and cadmium is known as one of the toxic heavy metals.

Both zinc and copper have been established as cofactors of a great number of enzymes, such as superoxide dimutase *(2,3),* involved in cell protection against oxidative stress *(4,5).* Several epidemiological studies have found a direct link between Zn and/or Cu deficiency and carcinogenesis *(5–11).*

Selenium is an essential component of glutathione peroxidase (GSH-Px). This antioxidant enzyme system protects the cell membrane against free-radical damage by reducing hydrogen peroxide and other organic hydroperoxides, thus inhibiting lipid peroxidation and damage to the cell. Trauma to the body causes an upsurge of free radicals and inflammation, increasing the need for selenium as an antioxidant *(2).*

Iron is one of the most important essential elements. A great number of human diseases are related to iron deficiency or to disorders of its metabolism. Nickel may be involved in facilitating intestinal absorption of ferric iron. Because of the iron–nickel interaction, the metabolism of nickel may be altered in individuals with higher iron or copper levels. It is also known that occupational exposure to nickel may occur simultaneously with chromium. Bruce-Robertson et al. *(12)* studied the harmful effects of Cr (VI) in blood vessels, nerves, and other tissues.

Cadmium exposure occurs exclusively by occupational exposure (e.g., in miners or metal workers). Cigarette smoking is also another known source of Cd exposure *(13).* High levels of cadmium result in gonadal tissue damage in experimental animals and sterility in both sexes. Cadmium-induced vascular changes in ovaries lead to sterility, irrespective of the mode of exposure *(14).*

In this work, we present results that may be used as reference values for copper, iron, zinc, chromium, selenium, nickel, and cadmium in the cervical mucus of healthy women. Some relationships between these elements and age are also reported.

MATERIALS AND METHODS

Forty-five healthy women were selected for the study and separated to four group (30–40 yr, $n = 7$; 41–50 yr, $n = 19$; 51–60 yr, $n = 14$; >61 yr, $n = 5$).

Table 1
Detection Limits, Precision, and Recoveries of the Analytical Procedure

Element	Detection limit (pg)	Precision (CV%)	Recovery (%)
Copper	6.40		
7.5µg/L		3.39	87.72
30.0µg/L		1.16	91.80
Iron	7.20		
5.0µg/L		4.71	108.58
20.0µg/L		4.86	106.15
Zinc	0.98		
5.0µg/L		5.78	90.37
20.0µg/L		4.92	91.22
Cadmium	2.04		
0.5µg/L		1.64	95.21
2.0µg/L		1.02	90.57
Chromium	0.18		
2.5µg/L		1.95	98.54
10.0µg/L		1.98	96.51
Selenium	17.50		
2.5µg/L		2.81	92.71
10.0µg/L		3.11	94.26
Nickel	12.80		
2.5µg/L		1.76	112.50
10.0µg/L		4.43	107.69

They were not taking any medications and none had a history of cancer or of precancerous lesions to the cervix. The cervical mucus samples were collected during the follicular phase of their menstrual cycle. The age range of the women was from 32 to 75 yr, with an average age of 49.6 ± 9.23 yr.

After cleaning the cervical tip with a cotton pad, a cervical mucus specimen was taken by introducing a tuberculin syringe without the needle into the external opening of the cervix. The specimen was immediately transferred to a small plastic vial and stored at –20°C until use.

Each sample of cervical mucus was added to 100 µL concentrated nitric acid (Suprapur, Merck) and digested at room temperature for 24 h. After that, each sample was diluted to 1 mL with deionized water.

A Perkin-Elmer Zeeman 5100 PC Atomic Absorption Spectrophotometer provided with a HGA 600 Graphite Furnace Programmer, an AS-70 Autosampler and Auto Station 3600 from Pekin-Elmer (Norwalk, CT) was used to determine the elements in the samples. The detection limits, precision, and recovery values for copper, iron, zinc, selenium, cadmium, chromium, and nickel are given in Table 1. The reproducibility is acceptable because coefficients of variation for intra-assays are less than 10%.

All data were statistically analyzed using one-way analysis of variance (ANOVA), setting $p < 0.05$ for statistically significant differences. The

Table 2
Levels of Seven Elements in Cervical Mucus of Healthy Taiwanese Women

	Copper (mg/L)	Iron (mg/L)	Zinc (mg/L)	Cadmium (μg/L)	Chromium (μg/L)	Selenium (μg/L)	Nickel (μg/L)
Mean[1]	1.08	227.43	60.33	29.72	336.38	123.82	404.69
SD	1.14	401.22	71.68	40.43	773.87	105.42	889.64

[1] $n = 45$.

linear regression method was then used to analyze the levels of trace elements, which are significantly different for these four age groups.

RESULTS AND DISCUSSION

Table 2 shows the mean values of the seven elements studied in the cervical mucus of the 45 women. The mean values for copper, iron, zinc, cadmium, chromium, selenium, and nickel were 1.08 ± 1.14 μg/g, 227.43 ± 401.22 μg/g, 60.33 ± 71.68 μg/g, 29.72 ± 40.43 μg/kg, 336.38 ± 773.87 μg/kg, 123.82 ± 105.42 μg/kg, and 404.69 ± 889.64 μg/kg, respectively. The cervical mucus is difficult to obtain and the quantity of each sample is mostly less than 0.2 g. Although every woman is generally healthy, the conditions of the cervical mucus are different. This may result from different stages of menstrual cycle, individual difference, habits, or work.

The results of analyses with a breakdown with respect to age are shown in Table 3. The statistical analyses of the results by one-way ANOVA show that there is a significant age-dependent difference for selenium and nickel, $p < 0.05$. There were no age-related differences for copper, iron, zinc, cadmium, and chromium, $p > 0.05$.

The mean concentrations of selenium for each age group are shown in Table 3. These were significantly higher in the older age group. The linear regression curve for age versus selenium concentration is shown in Fig. 1. A significant correlation was found ($r = 0.23$, $p < 0.01$).

Because of the carcinogenic and infertility effects caused by certain metals acting on the cervical surface, there is an increasing interest in the study of the elemental composition of cervical mucus. A review of the literature on the subject of infertility reveals that several studies focus on the toxic effects of copper on sperm *(15–17)*. This may be relevant because the physiological mechanisms responsible for lowered copper in cervical mucus during the fertile phase may be important in permitting optimal sperm survival and transport *(18)*.

Table 3
Variations of the Element Concentration as a Function of Age

Element	Age Groups			
	30–40 (n=7)	41–50 (n=19)	51–60 (n=14)	>61 (n=5)
Copper (mg/L)	0.49±0.22	1.06±1.47	1.10±0.783	1.89±1.12
Iron (mg/L)	231.44±171.01	223.88±576.36	241.33±264.56	156.38±80.68
Zinc (mg/L)	79.22±82.68	36.48±56.31	78.40±86.87	73.96±52.60
Cadmium (µg/L)	18.18±14.90	30.35±56.20	33.95±29.77	31.60±15.77
Chromium (µg/L)	115.90±106.35	288.75±659.45	493.17±1130.3	387.05±525.81
Selenium (µg/L)	73.88±47.92	92.83±58.69	136.43±109.97	276.2±151.17
Nickel (µg/L)	1089.85± 1764.25	104.69±189.93	306.47±556.25	860.49± 1151.82

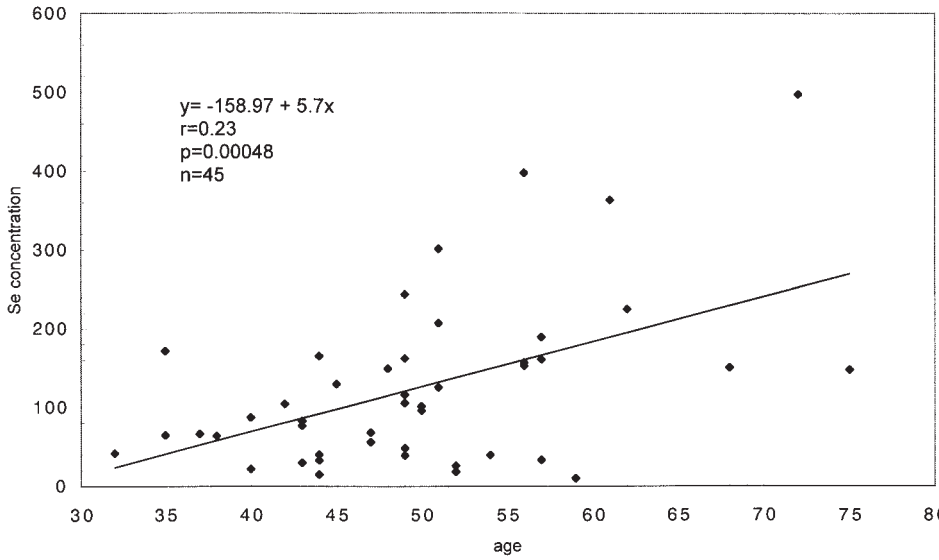

Fig. 1. Regression curve of age versus selenium in cervical mucus.

Few studies on cervical mucus mention other elements that are related to human diseases and tissue damage. Cervical glands excrete different elements, independently of its concentrations in serum *(19),* so it is absolutely necessary to establish the normal values of relevant elements in

such material to use in studies of abnormal conditions, where they can be used for comparison.

The normal values of copper, iron, zinc, selenium, cadmium, chromium, and nickel in the cervical mucus of healthy women and also in different age groups are reported in this work. It also demonstrates a significant progressive rise in the selenium content of cervical mucus with age. The factor influencing the rise of selenium may be a need for this element to protect the cervix against tumor formation, a condition that would increase by aging.

Several workers have reported an enhancement in the concentration of selenium in cancerous breast tissue and other tissues *(20–23).* Contrary to this, Vernie et al. *(24)* did not observe any significant changes in selenium levels of whole blood and plasma of breast cancer patients. Selenium is recognized as a versatile anticarcinogenic agent, possibly through the action of glutathione peroxidase. However, more research is necessary to clarify any role that selenium may play in cervical diseases, including cancer, that are related to lack of selenium protection.

In conclusion, the normal baseline levels for copper, iron, zinc, selenium, cadmium, chromium, and nickel are proposed for healthy Taiwanese women. The levels of selenium in cervical mucus of the 45 women significantly rise in the elder ones. The levels of the other elements had no significant differences related to age.

REFERENCES

1. L. Blasco, Cervical factor, in *Current Therapy of Infertility 1984–1985,* 3rd ed., C.-R. Garcia, L. Mastroianni, R. D. Amelar, and L. Dubin, eds., B. C. Decker Inc., Philadelphia, pp. 100–106 (1984).
2. L. Y. Danial, Selenium metabolism and bioavailability, *Biol. Trace Element Res.* **54,** 185–199 (1996).
3. P. E. Johnson, D. B. Milne, and G. L. Lykken, Effects of age and sex on copper absrption, biological half-life and status in humans, *Am. J. Clin. Nutr.* **56,** 917–925 (1992).
4. R. A. Disilvestro and G. P. Carlson, Effects of moderate copper deficiency on carbontetrachloride-induced hepatotoxicity in rats, *Proc. Soc. Exp. Biol. Med.* **197,** 32–35 (1991).
5. N. W. Solomons, Zinc and copper, in *Modern Nutrition in Health and Disease,* 8th ed., M. E. Shils and V. R. Young, eds., Lea & Febiger, Philadelphia, pp. 238–262 (1988).
6. Z. G. Jia, Analysis of serum levels of selenium, zinc, and copper in 132 patients with malignant tumors, *Chin. J. Prevent. Med.* **25,** 205–207 (1991).
7. E. L. Ma and Z. M. Jiang, Ion-exchange chromatography in simultaneous determination of serum copper and zinc levels in patients with cancer digestive tract, *Chinese Med. J.* **106,** 118–121 (1993).
8. D. J. Malvy, B. Burtschy, J. Arnaud, D. Sommelet, G. Leverger, L. Dostalova, et al., Serum beta-carotene and antioxidant micronutrients, in children with cancer, "Cancer in children and antioxidant micronutrients," The French Study Group, *Inst. J. Epidemiol.* **22,** 761–771 (1993).
9. K. Overvad, D. Y. Wang, J. Olsen, D. S. Allen, E. B. Thorling, and R. D. Bulbrook, Copper/zinc ratio in selected human mammary carcinogenesis: a case-cohort study, *Am. J. Epidemiol.* **137,** 409–414 (1993).

10. D. Yoshida, Y. Ikeda, and S. Nakazawa, Quantitative analysis of copper, zinc and copper/zinc ratio in selected human brain tumors, *J. Neurol. Oncol.* **16,** 109–115 (1993).
11. I. Yuel, F. Arpaci, A. Ozet, B. Doner, T. Jarayilanoglu, A. Sayar, et al., Serum copper and zinc levels and copper/zinc ratio in patients with breast cancer, *Biol. Trace Element Res.* **40,** 31–38 (1994).
12. K. N. Jeejeebhoy, R. C. Chu, E. B. Marliss, G. R. Greenberg, and A. Bruce-Robertson, Chromium deficiency, glucose intolerance, and neuropathy reversed by chromium supplementation, in a patient receiving long-term total parenteral nutrition, *Am. J. Clin. Nutr.* **30,** 531–538 (1977).
13. M. Vandi, Cadmium content in cigarettes, *Lancet* **7634,** 132 (1969).
14. A. D. Chiquoine and V. Suntzeff, Sensitivity of mammals to cadmium necrosis in the testis, *J. Rep. Fertil.* **10,** 455–460 (1965).
15. S. Saito, I. M. Bush, and W. F. Whitemore, Effects of certain metals and cheiating agents on rat and dog epididymal spermatozoan motility, *Fertil. Steril.* **18,** 517–529 (1967).
16. G. K. Oster, Reaction of metallic copper with biological substrates, *Nature* **134,** 153–154 (1971).
17. K. Loewit, Immobilization of human spermatozoa with iron. Basis for a new contraceptive, *Contraception* **2,** 219–224 (1971).
18. J. Zipper, H. Tatum, L. Pastene, M. Medel, and M. Rivera, Metallic copper as an intrauterine contraceptive adjunt to the "T" device, *Am. J. Obestet. Gynec.* **105,** 1274–1278 (1969).
19. K. N. von Kaulla, J. K. Aikawa, P. D. Bruns, and W. T. Wikle, Secretory function of the human uterine cervix, *Fertil. Steril.* **8,** 444–454 (1957).
20. I. L. Mulay, R. Ray, B. E. Knox, N. H. Suhr, and W. E. Dalaney, Trace-metal analysis of cancerous and noncancerous human tissues, *J. Natl. Cancer Inst.* **47,** 1–13 (1971).
21. A. E. Schwartz, G. W. Leddicotte, R. W. Fink, and E. W. Friedman, Trace elements in noraml and malignant human breast tissue, *Sugery* **76,** 325–329 (1974).
22. P. M. Santoliquido, H. W. Southwick, and J. H. Oliwin, Trace metal levels in cancer of the breast, *Surg. Gynecol. Obstet.* **142,** 65–70 (1976).
23. S. L. Rizk and H. H. Sky-Pack, Comparison between concentrations of trace elements in normal and neoplastic human breast tissue, *Cancer Res.* **44,** 5390–5394 (1984).
24. L. N. Vernie, J. J. Goeij, C. Zegers, M. Vries, G. S. Baldew, and J. G. McVie, Cisplatin-induced changes of selenium levels and glutathione peroxidase activities in blood of testis tumor patients, *Cancer Lett.* **40,** 83–91 (1988).