ระดับสังกะสี ทองแดง และโครเมียมในซีรัมของคนปกติ และผู้ป่วยมะเร็งแผนกศัลยกรรมโรงพยาบาลศิริราช

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
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SERUM ZINC, COPPER, AND CHROMIUM CONCENTRATION IN NORMAL SUBJECTS AND SURGICAL PATIENTS WITH CANCER IN SIRIRAJ HOSPITAL

Miss Nualnit Wichien

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นวลนิตย์ วิเชียร : ระดับสังกะสี ทองแดง และโครเมียมในซีรัมของคนปกติ และผู้ป่วยมะเร็งแผนกศัลยกรรมโรงพยาบาลศิริราช (SERUM ZINC, COPPER, AND CHROMIUM CONCENTRATION IN NORMAL SUBJECTS AND SURGICAL PATIENTS WITH CANCER IN SIRIRAJ HOSPITAL) อ. ที่ปรึกษา : รศ. ดร. อรอนงค์ กังสดาลอำไพ, อ. ที่ปรึกษาร่วม : ผศ. นพ. ธัญเดช นิมมานวุฒิพงษ์, 133 หน้า. ISBN 974-13-0946-5.

สังกะสี ทองแดง และโครเมียมเป็นแร่ธาตุปริมาณเล็กน้อยที่มีความจำเป็นต่อร่างกาย การศึกษานี้ได้วัดระดับสังกะสี ทองแดง และโครเมียมในซีรัมของคนปกติ 60 คน และผู้ป่วยมะเร็งแผนกศัลยกรรมโรงพยาบาลศิริราช 44 คน โดยใช้เครื่องสเปกโทรโฟโตมิเตอร์วัดการดูดกลืนแสงโดยอะตอม พบว่าระดับสังกะสี ทองแดง และโครเมียมในซีรัมของคนปกติมีค่า 84.58 ± 16.21 ไมโครกรัมต่อเดซิลิตร 89.08 ± 16.94 ไมโครกรัมต่อเดซิลิตร และ 0.47 ± 0.15 ไมโครกรัมต่อลิตร ตามลำดับ ในผู้ป่วยมะเร็งพบว่า ระดับสังกะสี ทองแดง และโครเมียมในซีรัมก่อนการผ่าตัดมีค่า 86.58 ± 46.97 ไมโครกรัมต่อเดซิลิตร 152.95 ± 51.81 ไมโครกรัมต่อเดซิลิตร และ 0.26 ± 0.14 ไมโครกรัมต่อลิตร ตามลำดับ ภายหลังการผ่าตัด 1 วัน มีค่า 62.70 ± 52.46 ไมโครกรัมต่อเดซิลิตร 128.35 ± 44.68 ไมโครกรัมต่อเดซิลิตร และ 0.14 ± 0.08 ไมโครกรัมต่อลิตร ตามลำดับ และภายหลังการ ผ่าตัด 7 วัน มีค่า 82.70 ± 62.49 ไมโครกรัมต่อเดซิลิตร 144.38 ± 42.60 ไมโครกรัมต่อเดซิลิตร และ 0.21 ± 0.10 ไมโครกรัมต่อลิตร ตามลำดับ เมื่อแบ่งผู้ป่วยมะเร็งออกเป็น 3 กลุ่มตามสภาวะโรค พบว่าระดับสังกะสีของผู้ป่วยในกลุ่มที่ 1, 2 และ 3 มีค่าเป็น 94.16 ± 53.12, 95.92 ± 37.64 และ 66.88 ± 42.13 ไมโครกรัมต่อเดซิลิตร ตามลำดับ ระดับทองแดงของผู้ป่วยในกลุ่มที่ 1, 2 และ 3 มีค่าเป็น 127.63 ± 31.35, 137.08 ± 38.70 และ 204.62 ±51.10 ไมโครกรัมต่อเดซิลิตร ตามลำดับ และระดับโครเมียมของผู้ป่วยในกลุ่มที่ 1, 2 และ 3 มีค่าเป็น 0.29 ± 0.14, 0.23 ± 0.15 และ 0.25 ± 0.12 ไมโครกรัมต่อลิตร ตามลำดับ เมื่อแบ่งผู้ป่วยมะเร็งออกเป็น 3 กลุ่มตามความรุนแรงของการผ่าตัด พบว่าระดับสังกะสีของผู้ป่วยในกลุ่มที่ 1, 2 และ 3 หลังผ่าตัด 1 วัน มีค่าเป็น 65.45 ± 38.55, 64.95 ± 60.51 และ 56.25 ± 51.84 ไมโครกรัมต่อเดซิลิตร ตามลำดับ ระดับทองแดงของผู้ป่วยในกลุ่มที่ 1, 2 และ 3 หลังผ่าตัด 1 วันมีค่าเป็น 142.95 ± 33.70, 140.95 ± 45.25 และ 92.92 ± 34.11 ไมโครกรัมต่อเดซิลิตร ตามลำดับ และระดับโครเมียมของผู้ป่วยในกลุ่มที่ 1, 2 และ 3 หลังผ่าตัด 1 วันมีค่าเป็น 0.11 ± 0.05, 0.16 ± 0.09 และ 0.14 ± 0.08 ไมโครกรัมต่อลิตร ตามลำดับ

ผลการศึกษาพบว่า ระดับสังกะสีในซีรัมของผู้ป่วยมะเร็ง ไม่แตกต่างจากคนปกติอย่างมีนัยสำคัญที่ระดับความเชื่อมั่นร้อยละ 95 อย่างไรก็ตามระดับสังกะสีนี้จะมีค่าต่ำที่สุดในผู้ป่วยมะเร็งกลุ่มที่ 3 ได้แก่ผู้ป่วยมะเร็งของตับ ท่อน้ำดี และตับอ่อน ระดับทองแดงในซีรัมของผู้ป่วยมะเร็งทุกกลุ่มมากกว่าคนปกติอย่างมีนัยสำคัญ ส่วนระดับโครเมียมในซีรัมของผู้ป่วยมะเร็งทุกกลุ่มน้อยกว่าคนปกติอย่างมีนัยสำคัญ ระดับสังกะสีในซีรัมภายหลังการผ่าตัด 1 วันในผู้ป่วยกลุ่มผ่าตัดที่ 1 และ 3 น้อยกว่าก่อนการผ่าตัดอย่างมีนัยสำคัญที่ระดับความเชื่อมั่นร้อยละ 95 ระดับทองแดงและโครเมียมในซีรัมภายหลังการผ่าตัด 1 วันในผู้ป่วยทุกกลุ่มผ่าตัด น้อยกว่าก่อนการผ่าตัดอย่างมีนัยสำคัญ

ภาควิชา……..อาหารเคมี……... ลายมือชื่อนิสิต……..

สาขาวิชา……อาหารเคมีและโภชนศาสตร์ทางการแพทย์…. ลายมือชื่ออาจารย์ที่ปรึกษา……

ปีการศึกษา….2543……. ลายมือชื่ออาจารย์ที่ปรึกษาร่วม……..

4276568033 : MAJOR FOOD CHEMISTRY
KEY WORD: SERUM ZINC / SERUM COPPER / SERUM CHROMIUM / TRACE ELEMENT / CANCER / SURGERY
NUALNIT WICHIEN : SERUM ZINC, COPPER, AND CHROMIUM CONCENTRATION IN NORMAL SUBJECTS AND SURGICAL PATIENTS WITH CANCER IN SIRIRAJ HOSPITAL. THESIS ADVISOR : ASSOC. PROF. ORANONG KANGSADALAMPAI, Ph. D., THESIS COADVISOR : ASSIST. PROF. THANYADEJ NIMMANWUDIPONG, MD. 133 pp. ISBN 974-13-0946-5.

Zinc, copper, and chromium are essential trace elements. In this study, serum zinc, copper, and chromium concentrations were determined in 60 healthy subjects and 44 surgical patients with gastrointestinal cancer by atomic absorption spectrophotometry (AAS). It was found that serum zinc, copper, and chromium in healthy subjects were 84.58 ± 16.21 μg/dl, 89.08 ± 16.94 μg/dl, and 0.47 ± 0.15 μg/l, respectively. Serum zinc, copper, and chromium of the entire population of cancer patients before operation were 86.58 ± 46.97 μg/dl, 152.95 ± 51.81 μg/dl, and 0.26 ± 0.14 μg/l, respectively. On the first day after operation, their levels were 62.70 ± 52.46 μg/dl, 128.35 ± 44.68 μg/dl, and 0.14 ± 0.08 μg/l, respectively. Seven days after operation, serum zinc, copper, and chromium were 82.70 ± 62.49 μg/dl, 144.38 ± 42.60 μg/dl, and 0.21 ± 0.10 μg/l, respectively. The cancer patients were categorized into 3 groups according to aggressiveness and prognosis of the disease. Serum zinc of patients in group 1, 2, and 3 were 94.16 ± 53.12 μg/dl, 95.92 ± 37.64 μg/dl, and 66.88 ± 42.13 μg/dl, respectively. Serum copper of patients in group 1, 2, and 3 were 127.63 ± 31.35 μg/dl, 137.08 ± 38.70 μg/dl, and 204.62 ± 51.10 μg/dl, respectively. Serum chromium of patients in group 1, 2, and 3 were 0.29 ± 0.14 μg/l, 0.23 ± 0.15 μg/l, and 0.25 ± 0.12 μg/l, respectively. The cancer patients were also categorized into 3 groups according to the extent of surgery. Serum zinc on the first day after operation (Day 1) of patients in group 1, 2, and 3 were 65.45 ± 38.55 μg/dl, 64.95 ± 60.51 μg/dl, and 56.25 ± 51.84 μg/dl, respectively. Serum copper on Day 1 of patients in group 1, 2, and 3 were 142.95 ± 33.70 μg/dl, 140.95 ± 45.25 μg/dl, and 92.92 ± 34.11 μg/dl, respectively. Serum chromium on Day 1 of patients in group 1, 2, and 3 were 0.11 ± 0.05 μg/l, 0.16 ± 0.09 μg/l, and 0.14 ± 0.08 μg/l, respectively.

The results demonstrated that serum zinc of cancer patients was not significant different from normal subjects ($p > 0.05$). However, the lowest concentration was found in patients of group 3 (hepatocellular carcinoma, cholangiocarcinoma, cancer head of pancreas, and cancer ampulla of vater). Serum copper of cancer patients in all disease groups were significantly higher than normal subjects ($p < 0.05$) and highest in patients of groups 3, whereas serum chromium of cancer patients in all disease groups were significantly lower than normal subjects ($p < 0.05$). Serum zinc on the first day after operation (Day 1) of patients in surgery group 1 and 3 were significantly lower than the day before operation (Day 0) ($p < 0.05$). Serum copper and chromium on Day 1 in all surgery groups of patients

Department…..Food Chemistry…… Student Signature…….
Field of Study Food Chemistry and Medical Nutrition Advisor Signature…….
Academic Year…2000…… Co-advisor Signature……

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LIST OF ABBREVIATIONS

AAS	=	Atomic absorption spectrophotometry
ACTH	=	Adrenocorticotrophic hormone
AE	=	Acrodermatitis enteropathica
A.N.	=	Admission number
AR	=	Analytical reagent
B.C.	=	Before Christ
BMI	=	Body mass index
cm	=	Centimeter (s)
CSF	=	Cerebrospinal fluid
°C	=	degree celcius
dl	=	deciliter (s)
DNA	=	Deoxyribonucleic acid
DTPA	=	Diethylene triamine penta-acetate
e.g.	=	exampli gratia (for example)
et al.	=	et alii (and others)
g	=	gram (s)
HDL	=	high density lipoprotein
H.N.	=	Hospital number
hr	=	hour (s)
i.e.	=	id est (that is)
kg	=	kilogram (s)

l	=	liter (s)
mA	=	Milliampare (s)
mg	=	milligram (s)
min	=	minute (s)
ml	=	milliliter (s)
mRNA	=	Messenger ribonucleic acid
N	=	Normal
nm	=	Nanometer (s)
No.	=	Number
PEM	=	protein energy malnutrition
RDA	=	Recommended Dietary Allowances
RNA	=	Ribonucleic acid
rpm	=	Revolution (s) per minute
SD	=	standard deviation
S/P	=	status post
TPN	=	Total parenteral nutrition
VS	=	Versus
μg	=	Microgram (s)
μl	=	Microliter (s)
%	=	Percentage
>	=	more than
<	=	less than

CHAPTER I

INTRODUCTION

Hospitalized patients are usually in stress condition. Some patients may develop malnutrition because of disease such as gastrointestinal disease, cancer, complication of illness, and infection after surgery. Chiolero, Revelly, and Tappy (1997) indicated that the development of malnutrition was often rapid in patients with sepsis and surgical injury. In such patients, hormonal and nonhormonal mediators were released, inducing complex metabolic changes. The patients have increased morbidity and mortality rates, and required longer hospitalization. Thus, nutritional support is an important supportive treatment in management of these patients, particularly in those with surgical injury or prolonged hospitalization. This should be done as soon as the patients are admitted in order to prevent, correct or alleviate their inadequate nutritional status and also improve a previously malnourished individual.

Generally, man requires six groups of essential nutrients, i.e., carbohydrates, fats, proteins, vitamins, minerals, and water. Minerals required by man may be divided into two groups based on their quantitative requirements: macrominerals and trace minerals. Trace minerals are inorganic elements that regulate numerous metabolic process in the body. Nine trace elements of biologic importance definitely essential in humans are iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), selenium (Se), chromium (Cr), cobalt (Co), iodine (I), and molybdenum (Mo) (Solomons, 1986). Depletion of these substances causes functional, biochemical or structural abnormalities in tissues. Patients with

gastrointestinal cancer often present the symptoms of dysphagia, gastrointestinal obstruction, nausea, vomiting, and decreased production of digestive secretions (Frankmann, 1996). The patients may develop trace element deficiency. The acute-phase response to injury or infection is also associated with alteration in dynamics of many trace elements (Shenkin, 1995). Hoffman (1985) showed that mineral requirements in cancer patients were different from other patients. To prevent the deficit of these nutrients, minerals should be adequately supplied together with other nutrients.

Zinc (Zn), an essential trace mineral, is important for protein synthesis, maintenance of cell membrane stability and function, gene expression, and cell-mediated immunity. Zinc is known for its ability to form many types of metalloenzymes. Some of the widely known zinc-containing enzymes include alcohol dehydrogenase, alkaline phosphatase, angiotensin-converting enzyme, carbonic anhydrase, DNA polymerase, and Cu/Zn superoxide dismutase (Braunschweig, 1998). Zinc deficiency is associated with certain patient subpopulations such as elderly, alcoholics, postsurgical, burn patients, and those with malabsorption syndrome. Zinc deficiency typically is manifested as blunting of taste and smell, alopecia, skin rash on the face, diarrhea, growth retardation, delayed sexual development, delayed wound healing, and impaired immune function (Braunschweig, 1998; Herr, 1994; Okada et al., 1976; Prasad, 1991). Many investigators indicated a decrease serum zinc concentration in patients with gastrointestinal, hepatic, infectious, cardiovascular, including malignant diseases (C. Pramoolsinsap et al., 1994; Fernandez-Banares et al., 1990; Sriwatana Songchitsomboon et al., 1999). Serum or plasma zinc levels in patients with cancer have been observed to be lower than those

in normal (C. Pramoolsinsap et al., 1994; Davies, Musa, and Dormandy, 1968; Diez et al., 1989; Gupta et al., 1993; Inutsuka and Araki, 1978; Issell et al., 1981; Lightman et al., 1986; Mellow et al., 1983; Poo et al., 1997; Stefanini, 1999; Vikua Skulchan et al., 1987).

Copper (Cu) is important for the function of numerous oxidative enzymes, known as metalloenzymes. Metalloenzymes containing copper are involved in oxygen-using reactions. The most abundant copper enzyme is cytochrome oxidase, the iron-containing terminal component of the electron transport chain in all human cells. Cu/Zn superoxide dismutase is an important scavenger of superoxide anions. Some other of the common metalloenzymes include monoamine oxidase, diamine oxidase, and dopamine β-hydroxylase (Braunschweig, 1998; Herr, 1994). These enzymes are integral to metabolic functions such as erythropoiesis, leukopoiesis, oxidative phosphorylation, catecholamine metabolism, antioxidant protection, and the maintenance of immunocompetence (Herr, 1994; Turnlund, 1994). Copper deficiency is characterized by hypochromic microcytic anemia, neutropenia, increased risk of myocardial disease, increased serum cholesterol, and heartbeat irregularities (Braunschweig, 1998; Herr, 1994; Turnlund, 1994). Serum copper of patients with many types of cancer such as malignant tumors of digestive organs, colorectal cancer, hepatocellular carcinoma, breast cancer, and lung cancer were mostly elevated (C. Pramoolsinsap et al., 1994; Diez et al., 1989; Garofalo et al., 1980; Gupta et al., 1993; Inutsuka and Araki, 1978; Miatto et al., 1985; Poo et al., 1997).

Another essential trace element is chromium (Cr) which functions primarily to potentiate the action of insulin. Chromium is not associated with enzymes but instead acts as a coordinating compound in the control of glucose metabolism. It is thought to be the active component of the low-molecular-weight organic complex termed glucose tolerance factor, acts with insulin in promoting optimal glucose utilization. Chromium enhances the ability of insulin to bind to the insulin receptors on cell surfaces and to allow entry of glucose into the cell. Besides improving glucose control, glucose tolerance factor may lower serum cholesterol and triglyceride levels (Braunschweig, 1998; Herr, 1994). Chromium deficiency is known to result in decreased glucose tolerance, glucose tolerance factor, impairment of glucose metabolism (Okada et al., 1995), elevated serum glucose, cholesterol and triglyceride, weight loss, glucosuria and peripheral neuropathy. Further, with insulin dependent diabetic patients or with stressful events such as trauma, strenuous exercise, severe protein calorie malnutrition, pregnancy, lactation or infection, chromium losses are increased (Anderson, 1997; Herr, 1994). The concentration of chromium in cerebrospinal fluid of patients with malignant brain tumors was found to be depleted (El-Yazigi, Martin, and Siqueira, 1988).

Many studies pointed out that the serum copper level increased and the serum zinc level decreased in patients with various types of cancer, i.e., digestive (Inutsuka and Araki, 1978; Poo et al., 1997), esophageal (Mellow et al., 1983), laryngeal (Stefanini, 1999), head and neck (Westin et al., 1989), colorectal (Gupta et al., 1993), hepatic (C. Pramoolsinsap et al., 1994; Miatto et al., 1985), breast (Garofalo et al., 1980), lung (Diez et al., 1989; Issell et al., 1981), and ovary malignancies (Lightman et al., 1986). However, there are contradictory reports on the

alterations in serum zinc levels in patients with breast cancer (Cavallo et al., 1991; Margalioth, Schenker, and Chevion, 1983). Furthermore, only serum zinc in cancer patients was reported in Siriraj Hospital (Vikua Skulchan et al., 1987) and there are limited data on other trace elements in such patients with cancer, i.e., chromium (El-Yazigi et al., 1988). Previous studies of the influence of surgery on serum trace elements have investigated in a relatively small number of cases (Antila et al., 1990; Miatto et al., 1985). Therefore, it is worth interesting to investigate the changes in serum zinc, copper, and chromium concentration of surgical patients with cancer especially gastrointestinal cancer in Siriraj Hospital, and to study the influence of cancer conditions and surgery on these serum trace elements. This study may provide the guideline information in trace element supplementation to these patients in order to gain the quality of life of the patients.

The zinc, copper and chromium status can be assessed by determining the concentration of the trace elements in plasma, serum, erythrocytes, hair, urine, and metalloenzymes or related proteins. Chromium can be indirectly measured by monitoring blood glucose, insulin and glucose tolerance. This study, the serum zinc, copper, and chromium concentrations were determined by atomic absorption spectrophotometry. This method is preferred in the clinical laboratory because of its specificity, sensitivity, accuracy, precision, and simplicity (Milne, 1994; Milne and Johnson, 1993; Smith, Butrimovitz, and Purdy, 1979).

The objectives of the study

The purposes of this study were

1. To compare serum zinc, copper and chromium concentration in normal subjects and surgical patients with cancer in Siriraj Hospital
2. To study the effect of cancer conditions and surgery on serum zinc, copper and chromium concentration
3. To evaluate the needs for supplementation of these trace elements in surgical patients with cancer

Expectations

1. To know serum zinc, copper and chromium concentration in normal subjects and surgical patients with cancer in Siriraj Hospital
2. To know the effect of cancer conditions and surgery on serum zinc, copper and chromium concentration

To provide the guideline information for planning to supplement zinc, copper and chromium in surgical patients with cancer

CHAPTER II

LITERATURE REVIEW

Zinc

Zinc (Zn) was recognized as a distinct element in 1509. Evidence of its essentiality was demonstrated in animals in 1934. Because of its wide prevalence in foodstuffs, naturally occurring zinc deficiency was considered unlikely until 1955, when swine parakeratosis was shown to be a zinc-deficiency disease. Humans suffering from zinc deficiency was observed in malnourished Chinese patients during World War II who had low concentrations of plasma zinc. In 1956, a conditioned zinc-deficiency syndrome in humans was demonstrated. Since 1961, when the endemic hypogonadism and dwarfism was suggested to be derived from zinc deficiency, there has been an increasing appreciation of the magnitude of both the clinical and the public health significance of zinc-deficiency states (King and Keen, 1994).

1. Functions

Zinc is an essential constituent of more than 200 metalloenzymes. Some of the widely known zinc-containing enzymes include alcohol dehydrogenase, alkaline phosphatase, angiotensin-converting enzyme, carbonic anhydrase, DNA polymerase, and Cu/Zn superoxide dismutase (Braunschweig, 1998). It is involved in most of the control metabolic pathways, including metabolism of proteins, fats, and carbohydrates. Zinc acts as a stabilizer of polysomes during protein synthesis (King

and Keen, 1994). It stabilizes the structure of certain DNA-binding proteins that are involved in the transcription of DNA to RNA. It is also involved in the immune system, maintaining the stability of lipids in cell membranes, and promoting of wound healing (Braunschweig, 1998).

2. Zinc in the Human Body

Zinc is present in all organs, tissues, fluids, and secretions of the body. Zinc is found primarily in hair, bones, liver, kidneys, skeletal muscle, and skin. The zinc concentration and content of various tissues are present in Table 1.

3. Absorption

Zinc is absorbed all along the small intestine; only small amounts are absorbed in the stomach and large intestine. Considering the length and surface area of the various segments of the small bowel, the transit time of digestion, and the endogenous secretion of zinc, most of the element is probably absorbed in the jejunum (King and Keen, 1994). In general, approximately 20 – 40 % of ingested zinc is absorbed by healthy persons (Braunschweig, 1998).

During the process of digesting a meal, digestive enzymes release dietary zinc from food matrices and endogenous zinc from various binding ligands. As such, this free zinc is able to form coordination complexes with various exogenous and endogenous ligands, such as amino acids, phosphates, and other organic acids. Histidine and cysteine are the preferred amino acid ligands and enhance zinc absorbability by forming stable complexes with zinc (King and Keen, 1994).

Table 1. Approximate zinc content of major organs and tissues in a normal adult man (70 kg) (King and Keen, 1994)

Tissue	Approximate zinc concentration (μg/g)	Total zinc content in the body (g)
Skeletal muscle	51	1.53
Bone	100	0.77
Skin	32	0.16
Liver	58	0.13
Brain	11	0.04
Kidneys	55	0.02
Heart	23	0.01
Hair	150	< 0.01
Blood plasma	1	< 0.01

Age, body size, zinc level in the diet, nutritional status, and the presence of calcium, phosphate, fiber, phytate, other chelating agents and vitamin D are the major factors affecting zinc absorption. Phytate (inositol hexaphosphate), the major inhibitor of zinc absorption, is present in all cereals and most vegetables. It can bind zinc and reduce the bioavailability of zinc (O'Dell, 1969; Reinhold et al., 1973).

Therefore, zinc is more available for absorption from animal foods than from plant sources. The presence of other divalent metal ions, such as iron, may compete with zinc for mucosal cell binding sites (King and Keen, 1994).

Once enters mucosal cells, zinc is bound to metallothionein, a low-molecular weight protein with a high cysteine content that is responsible for homeostatic regulation of zinc absorption (Braunschweig, 1998). The zinc then moves with metallothionein from the intestinal cell to the blood and is bound to albumin for transport (Herr, 1994).

4. Transportation

After being absorbed, zinc is transported in the blood bound to the carrier proteins, albumin (66 %) or α-2-macroglobulin (32 %) and the amino acids histidine and cysteine (1 %) (Braunschweig, 1998). These loosely bound albumin and amino acid fractions of circulating zinc provide the transport and delivery of zinc to various tissues (King and Keen, 1994). The total amount of zinc present in the major tissues is much larger than that present in plasma. A relatively small variation in the zinc tissue content, such as the liver, can have dramatic effects on plasma zinc. For example, an increase of liver zinc by 1 % could cause a 40 % decline in plasma zinc. All absorbed zinc passes through the plasma to the tissues, therefore, the flux of zinc through the plasma must be rapid to maintain relatively constant plasma concentrations (King and Keen, 1994).

The elimination of absorbed zinc from the body was best explained by a two-component model (King and Keen, 1994). The initial rapid phase has a half-life in humans of 12.5 days, and a slower turnover phase has a half-life value of about 300 days. The initial rapid half-life primarily represents liver uptake of circulating zinc and its release. The slower turnover rate reflects differing rates of zinc turnover in various tissues other than liver. Figure 1 illustrates the metabolism of zinc.

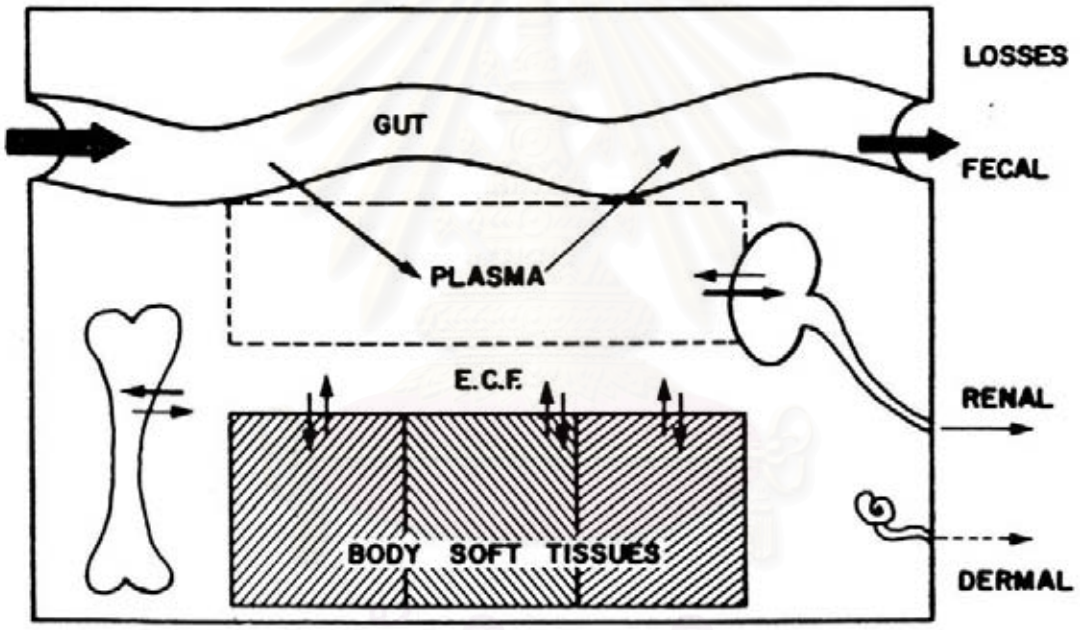

Figure 1. Schematic representation of the metabolism of zinc in mammals (King and Keen, 1994)

5. Excretion

Excretion of zinc occurs principally via the gastrointestinal tract, from fecal losses, and the remaining zinc is excreted in the urine (Braunschweig, 1998). In humans, endogenous fecal losses may range from < 1 mg/day with extremely low intakes to over 5 mg/day with extremely high intakes (King and Keen, 1994).

Normally, about 400 to 600 μg of zinc is excreted daily in the urine. Urinary zinc arises largely from the ultrafilterable portion of the plasma zinc. Dietary zinc only influences urinary losses if the intake is extremely low or extremely high. Under basal conditions, up to 95 % of the filtered zinc is reabsorbed in the distal parts of the renal tubule. Catabolic states, such as those resulting from severe burns, major surgery or other trauma, and total starvation, cause clinically significant increases in urinary zinc losses. Surface losses through desquamation of skin, outgrowth of hair, and sweat contribute up to 1 mg of zinc daily (King and Keen, 1994).

6. Dietary Sources

Foods differ widely in their zinc content. The zinc concentrations range from 0.02 mg/100 g for egg white to 1 mg/100 g for chicken meat to 75 mg/100 g for oysters. Shellfish, beef, and other red meats are good zinc sources. Whole-grain cereals are relatively rich in total zinc. Nuts and legumes are relatively good plant sources of zinc. Plant zinc concentrations may be increased if grown in zinc-rich soil (King and Keen, 1994).

7. Requirements and Recommended Intakes

Human nutrient requirements are generally based on one of the following criteria: (1) the amount required to support balance; (2) the amount required to replace endogenous loss; or (3) the amount needed to maintain normal function. Zinc requirements are generally based on the amount needed to support balance or to replace endogenous losses (King and Keen, 1994). The 1989 Recommended Dietary Allowance (RDA) for zinc is shown in Appendix A, Table 10.

8. Zinc Deficiency

Growth retardation, delayed sexual maturation, hypogonadism, alopecia, immune deficiencies, mental lethargy, night blindness, hypogeusia, delayed wound healing, and impaired appetite and food intake were the pathological conditions in man that appear to be the consequence of inadequate zinc nutrition (Prasad, 1991; Prasad, 1995).

The dermatological manifestation of severe zinc deficiency include progressive bullous-pustular dermatitis of the extremities, the oral, anal, and genital areas; combined with paronychia and generalized alopecia as seen in Acrodermatitis Enteropathica (AE) (Prasad, 1991). AE is a rare, inherited autosomal recessive disease of skin/gastrointestinal tract disorder resulting in reduced intestinal zinc absorption (Herr, 1994). Skin lesions in patients receiving prolonged intravenous hyperalimentation were observed (Okada et al., 1976). These cutaneous manifestations frequently were accompanied by abdominal symptoms resembling those of AE. However, skin eruptions disappeared promptly after administration of zinc.

Zinc level has been observed to vary in various diseases. Many investigators have demonstrated a decrease serum zinc concentration in patients with gastrointestinal, hepatic, pulmonary, renal, infectious, cardiovascular, including malignant diseases (C. Pramoolsinsap et al., 1994; Fernandez-Banares et al., 1990; Sriwatana Songchitsomboon et al., 1999).

Causes of zinc deficiency

(1) Increased requirements of zinc: In growing infants, children, adolescents, elderly, pregnant and lactating women, the potential for zinc deficiency may be increased. This is especially true if the dietary supply is inadequate (Herr, 1994; King and Keen, 1994).

(2) Increased losses of zinc: Patients with gastrointestinal fistula, diarrhea, diabetes mellitus, renal failure, alcoholism, alcoholic with liver disease, infection, inflammation, malignant disease, stress and surgery often have excessive zinc losses (Herr, 1994; King and Keen, 1994).

(3) Inadequate zinc intake: Individuals with conditions of semistarvation such as anorexia and protein-energy malnutrition (PEM), poor digestibility and absorbability cause by high levels of dietary phytate and fiber often have an insufficient zinc intake (King and Keen, 1994).

(4) Decreased absorption of zinc: In diseases of gastrointestinal tract such as Crohn's disease, inflammatory bowel disease, sprue, short bowel syndrome, jejunoileal bypass surgery, and Acrodermatitis Enteropathica, the malabsorption of zinc has been observed (King and Keen, 1994).

(5) Some chelating agents and drugs: D-penicillamine, Diethylene triamine penta-acetate (DTPA), Sodium valproate, Corticosteroids, Estrogens and Oral contraceptives have been shown to produce zinc depletion (Milne, 1994).

9. Zinc Toxicity

9.1 Acute toxicity

Although rare, incidences of acute zinc toxicity in humans resulting from high intakes of zinc have been reported. Typical signs of acute zinc toxicosis include epigastric pain, diarrhea, nausea, vomiting, dehydration, electrolyte imbalance, dizziness, and muscular incoordination. Doses in excess of 200 mg a day are typically emetic. A fatal outcome occurred when inadvertently given 1.5 g of zinc intravenously over a 3-day period (King and Keen, 1994).

9.2 Chronic toxicity

The major consequence of the long-term ingestion of excessive zinc supplements is the induction of a secondary copper deficiency. One explanation is that a high intake of zinc induces the synthesis of the copper-binding ligand, metallothionein, in the mucosal cell. This protein sequesters copper, making it unavailable for serosal transfer and thus decreases copper absorption (King and Keen, 1994). Prasad et al. (1978) reported that hypocupremia associated with microcytosis

and relative neutropenia occurred in sickle cell anemia adult received zinc orally 150 mg daily for 2 years. This complication was easily corrected by copper supplementation. The long-term consumption of zinc supplements in excess of 150 mg per day has also been reported to result in low serum high density lipoprotein-cholesterol (HDL-cholesterol) levels, gastric erosion, and depressed immune function (King and Keen, 1994).

10. Assessment of Zinc Status

Approaches for assessing nutritional status in the laboratory involve the measurement of 2 indices (King and Keen, 1994);

10.1 Static indices

Concentration of the nutrient in tissues or fluids or measurement of metal-containing enzymes and proteins have been a measure of zinc status. Other static measures of zinc status are erythrocyte metallothionein, erythrocyte zinc, leukocyte zinc, hair zinc, and urinary zinc. The most popular techniques for the determination of zinc in biological specimens currently used include atomic absorption spectrophotometry (AAS) because of its specificity, sensitivity, accuracy, precision, and simplicity. (Milne, 1994; Milne and Johnson, 1993; Smith et al., 1979)

10.2 Functional indices

The most definitive index is isotopic measurements of the labile, or nutritionally available, zinc pool size. Many of the other functional tests are measurements of wound healing and nitrogen retention.

Factors affecting the determinated serum zinc concentration by atomic absorption spectrophotometer are present in Table 2.

Table 2. Factors affecting the determinated serum zinc concentration by atomic absorption spectrophotometer (Milne, 1994)

Changes	**Factors**
Serum zinc increased	Environmental contamination
	Hemolysis
Serum zinc decreased	Estrogens, Oral contraceptives
	Corticosteroids
	Infection, inflammation, stress, trauma, surgery
	Hypoalbuminemic condition, i.e., hepatic cirrhosis, malnutrition
	Pregnancy
	Alcoholic cirrhosis

Copper

Copper (Cu) has been used therapeutically since at least 400 B.C. Copper was identified as a normal constituent of blood and its toxicity was described in the late nineteenth century. By 1900, an anemia that could not be prevented by iron supplements had been observed in animals kept on a whole-milk diet. In 1928, this anemia in rats was responsive to iron only when copper supplements were also given (Turnlund, 1994).

Human disease was first linked to copper metabolism shortly after Wilson's disease was described in 1912, and long before the condition was recognized as an inborn error of metabolism in 1953. Menkes' disease, another genetic disorder, was described in 1962 and recognized as a disorder of copper absorption in 1972 (Turnlund, 1994).

1. Functions

Copper functions in vivo as a part of a number of proteins, including many important enzymes. The copper proteins known to be present in human beings are copper-containing enzymes, known as cuproenzymes (monoamine oxidase, tyramine oxidase, lysyl oxidase, ferroxidase I or ceruloplasmin, ferroxidase II, cytochrome oxidase, dopamine β-hydroxylase, Cu/Zn superoxide dismutase) and copper-binding proteins (metallothionein, albumin, blood clotting factor V) (Linder and Hazegh-Azam, 1996; Turnlund, 1994).

Many of the physiologic functions of copper can be deduced from reactions the cuproenzymes catalyze (Braunschweig, 1998; Turnlund, 1994).

1.1 Connective tissue formation

Copper is essential for cross-linking of collagen and elastin, which are required for the formation of strong, flexible connective tissue. Thus, it plays a role in bone formation, skeletal mineralization, and the integrity of the connective tissue in the heart and vascular system.

1.2 Iron metabolism

Ceruloplasmin and ferroxidase II oxidize ferrous iron, so it can be transported from the intestinal lumen and storage sites to sites of erythropoiesis. Copper may also required for the formation of normal bone marrow cells, necessary for the formation of red blood cells.

1.3 Central nervous system

Copper is required for the formation or maintenance of myelin, a protective layer covering neurons composed primarily of phospholipids. The role of cuproenzymes in catecholamine metabolism (the conversion of dopamine to norepinephrine by dopamine β-hydroxylase and the degradation of serotonin, norepinephrine, tyramine, and dopamine by monoamine oxidase) implies a function in normal neurotransmission.

1.4 Melanin pigment formation

The role of copper in the pigmentation of skin, hair, and eyes is related to the requirement for tyrosinase in melanin synthesis. Depigmentation of hair and skin is observed with copper deficiency in several animal species.

1.5 Other functions

Copper has a role in thermal regulation, cholesterol metabolism, glucose metabolism, immune function, cardiac function, and blood clotting through factor V.

2. Absorption

Copper is absorbed primarily in the small intestine, with a small amount absorbed in the stomach. Absorption is probably by a saturable, active transport mechanism at low levels of dietary copper and, at high levels of dietary copper, passive diffusion plays a role. Absorption may be regulated by the need for copper, with metallothionein in intestinal cells involved in the regulation (Linder and Hazegh-Azam, 1996; Turnlund, 1994).

Nutrients known to affect the bioavailability of copper when included in the diet in extreme amounts are iron, zinc, and ascorbic acid. Excessive iron in the form of inorganic iron salts decreased copper status and, in time, resulted in clinical signs of copper deficiency. When the diet contains excessive zinc over a sufficient period, the copper status has been impaired and the effect can be reversed by copper supplements. One explanation for this interaction is that high dietary zinc induces intestinal metallothionein. Copper does not induce metallothionein, but it has a stronger affinity for metallothionein than for zinc. It displaces zinc in intestinal metallothionein and is trapped. Ascorbic acid supplements may affect the copper status of humans. Daily ascorbic acid supplements of 1500 mg given to young men caused ceruloplasmin to decline and the oxidative activity of ceruloplasmin may be impaired (Linder and Hazegh-Azam, 1996; Turnlund, 1994).

3. Transportation and Regulation of Copper in the Body

Following being absorbed into the circulation, copper is transported bound primarily to albumin, and to transcuprein and low-molecular weight ligands. The newly absorbed copper disappears rapidly from the plasma. Most is taken up by the liver. Once in the liver, copper is incorporated into ceruloplasmin. Some is incorporated into metallothionein in the liver, particularly when copper intake is high. Copper is released from the liver into the blood bound to ceruloplasmin and delivered to cells throughout the body (Linder and Hazegh-Azam, 1996; Turnlund, 1994). Ceruloplasmin provides copper to the bone marrow for red and white blood cell production and/or donates copper for incorporation into various types of cuproenzymes (Herr, 1994).

4. Storage

The normal adult human body contains only approximately 50-120 mg of copper, very little compared to other trace elements such as iron and zinc. Ninety percent of which is found in liver, muscle and bone (Herr, 1994). The liver content is in large part related to its function as a storage organ for copper and also as the only site of synthesis and release of ceruloplasmin (Turnlund, 1994).

5. Excretion

The primary route of copper excretion is via bile into the gastrointestinal tract, it is then eliminated in the feces. Other routes of excretion contribute little to total copper losses. Healthy humans excrete only 10 to 30 μg of copper in the urine. Sweat and integumentary losses are usually less than 50 μg per day (Turnlund, 1994).

The copper metabolism initiating from absorption, transportation to excretion is depicted schematically in Figure 2.

6. Dietary Sources

The richest sources of dietary copper contain from 0.3 to over 2 mg/100 g. These include shellfish, nuts, seeds, legumes, and the bran and germ portions of grains, liver, and organ meats. Most grain products, fruits and vegetables such as dried fruits, mushrooms, tomatoes, bananas, grapes, and potatoes, and most meats have intermediate amounts of copper, from 0.1 to 0.3 mg/100 g. Other fruits and vegetables, chicken, many fish, and dairy products contain relatively low concentrations (less than 0.1 mg/100 g) of copper (Linder and Hazegh-Azam, 1996; Turnlund, 1994).

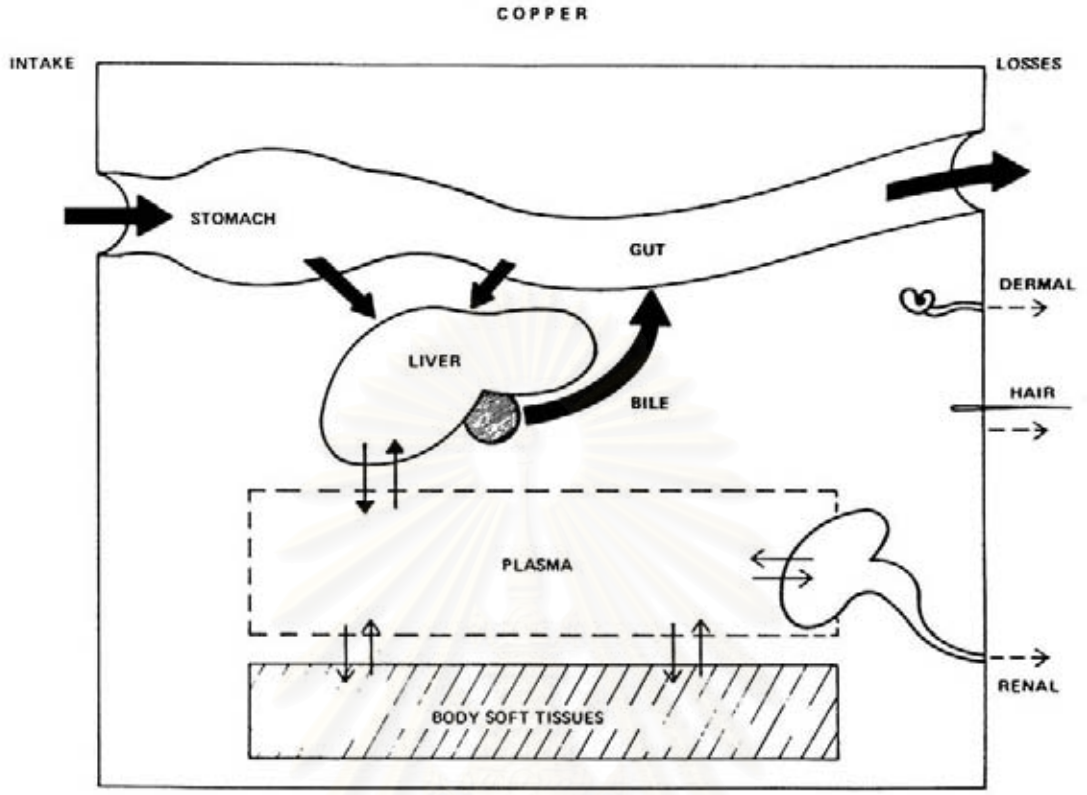

Figure 2. Schematic representation of the metabolism of copper in mammals (Turnlund, 1994)

7. Requirements and Recommended Intakes

The Estimated Adequate and Safe Daily Dietary Intakes of copper recommended by the National Research Council of the United States is present in Appendix A, Table 11.

8. Copper Deficiency

Copper deficiency is accompanied by hypocupremia and low ceruloplasmin levels. The most common clinical manifestations of copper deficiency are hypochromic microcytic anemia, leukopenia, and neutropenia (Herr, 1994). Osteoporosis is often observed when bones are still growing and may be accompanied by flaring of the metaphyses and fractures at the margins of the metaphyses. Possible manifestations, in addition to the features of severe deficiency, are conditions such as arthritis, arterial disease, depigmentation of hair, skin pallor, myocardial disease, and neurologic abnormalities. Diminished glucose tolerance, increased serum cholesterol, and heart beat irregularities have been linked to marginal copper intake (Milne, 1994; Turnlund, 1994).

Menkes' disease is a fatal x-linked disorder caused by a defect in intestinal copper absorption, increased urinary excretion and abnormal cellular transport of copper (Braunschweig, 1998; Herr, 1994). The syndrome is characterized by mental retardation, skin and hair depigmentation, defective arteries, seizures, and hypothermia. Furthermore, serum copper and ceruloplasmin levels are low in one individual with these symptoms (Turnlund, 1994).

Causes of copper deficiency

(1) Increased losses of copper: Patients with diarrhea, gastrointestinal fistulas, injury or one who received free amino acid solutions often have increased urinary copper losses (Turnlund, 1994).

(2) Decreased absorption of copper: In diseases of malabsorption such as Celiac disease and nontropical sprue increase the risk of copper depletion (Turnlund, 1994).

(3) Antacids and zinc treatment: Prolonged use of antacids and long-term therapy with very high doses of zinc in treatment of sickle cell anemia have resulted in hypocupremia and some manifestations of copper deficiency, microcytosis and neutropenia (Prasad et al., 1978).

(4) Inadequate copper intake: Patients receiving long-term parenteral nutrition without added copper usually develops copper deficiency (Turnlund, 1994).

9. Copper Toxicity

Copper excess may result from excessive copper intake, long-term exposure to hemodialysis solutions containing copper, or hereditary disorders of copper metabolism (i.e., Wilson' s disease) (Herr, 1994). Acute copper intoxication produces epigastric pain, nausea, vomiting, and diarrhea. Serious manifestations include oliguria, hepatic necrosis, vascular collapse, central nervous system damage, coma, and death (Braunschweig, 1998; Milne, 1994; Turnlund, 1994). Chronic excessive of copper intake results in liver cirrhosis. Tissue accumulation usually occurs only when the diet contains 200 - 500 times the normal amount of copper (Braunschweig, 1998).

Wilson' s disease is an autosomal recessive disease of copper storage. Copper accumulates in the liver, brain, kidneys, and corneas (Braunschweig, 1998; Turnlund, 1994). Urinary copper excretion is abnormally high, but ceruloplasmin values are usually low. These appear to be a defect in the catabolism and excretion of ceruloplamin and copper into the bile. Copper accumulation in the liver and brain results in neurologic damage and cirrhosis. Hepatitis, hemolytic crisis, and hepatic failure may ensure. Chelation therapy, usually using D-penicillamine is effective in reducing copper stores (Turnlund, 1994). A newer maintenance therapy is zinc which blocks absorption of copper in the intestine by inducing intestinal cell metallothionein (Brewer, 1995).

10. Assessment of Copper Status

Serum copper and ceruloplasmin concentrations have generally been considered the most reliable index for assessing copper status, but some consider red blood cell superoxide dismutase activity may be equally or more sensitive. Other indices of copper status are copper levels in erythrocyte, urine, hair, nails, or saliva. The most popular techniques for the determination of copper in biological specimens currently used include atomic absorption spectrophotometry (AAS) because of its specificity, sensitivity, accuracy, precision, and simplicity (Milne, 1994; Milne and Johnson, 1993; Smith et al., 1979).

Factors affecting the determinated serum copper concentration by atomic absorption spectrophotometer are present in Table 3.

Table 3. Factors affecting the determinated serum copper concentration by atomic absorption spectrophotometer (Herr, 1994; Milne, 1994; Milne and Johnson, 1993)

Changes	**Factors**
Serum copper increased	Environmental contamination
	Estrogens, Oral contraceptives
	Smoking
	Infection, inflammation, pregnancy
	Hematologic, coronary, cardiovascular, diabetic, malignant disease
Serum copper decreased	Impaired synthesis or release of ceruloplasmin
	Surgery
	D-penicillamine
	Corticosteroids

Chromium

The first suggestion that chromium might have biologic activity appeared in 1954. In 1959, trivalent chromium was identified as the active component of "glucose tolerance factor", which alleviated the impaired glucose tolerance in rats fed certain diets apparently inadequate in chromium. Between 1964 and 1968, the first reports indicated that chromium could affect glucose tolerance in humans. In these studies, mildly diabetic patients, or subjects with impaired glucose tolerance, received supplements of 150 to 200 μg chromium per day, the supplementation improved the impaired glucose tolerance of 40 to 50 % of these individuals. Subsequently, it was found that the chromium supplementation also decreased serum cholesterol and normalized the exaggerated insulin responses to glucose loads. Until 1977, chromium deficiency signs in a patient receiving total parenteral nutrition (TPN) were described. Shortly thereafter, other patients receiving TPN were found to exhibit abnormalities of glucose metabolism that were responsive to chromium supplementation (Milne, 1994; Nielsen, 1994).

1. Functions

Chromium is generally accepted as an essential nutrient that potentiates insulin action. Chromium is presumed to enhance the ability of insulin to bind to insulin receptors on cell surfaces and to allow entry of glucose into the cell. In this capacity, chromium influences carbohydrate, lipid, and protein metabolism (Braunschweig, 1998).

Some patients receiving TPN who exhibited signs of diabetes, including glucose intolerance, were refractory to insulin. After chromium supplementation, however, their diabetic symptoms were alleviated or their need for exogenous insulin was eliminated. This finding supports the concept that chromium has a biochemical function that affects the ability of the insulin receptor to interact with insulin (Milne, 1994; Nielsen, 1994).

2. Absorption

Chromium is absorbed in the upper small intestine. The mechanism of absorption of chromium from the intestine apparently involves processes other than simple diffusion (Milne, 1994; Nielsen, 1994).

3. Distribution

Following absorption, both transferrin and albumin are capable of binding absorbed chromium and transporting it as part of blood serum or plasma. It has been suggested that transferrin is the main binder of newly absorbed chromium, and albumin assumes the role of chromium acceptor and transporter of chromium if transferrin binding sites are unavailable. Other plasma proteins, including α- and β-globulins, and lipoproteins, bind chromium and thus may have a role in chromium metabolism (Milne, 1994; Nielsen, 1994).

4. Excretion

Absorbed inorganic trivalent chromium is excreted primarily through the kidney, with small amounts lost in hair, sweat, and bile (Milne, 1994; Nielsen, 1994). Normal healthy subjects excrete 0.22 μg per day via urine (Braunschweig, 1998). Stress conditions, i.e., strenuous exercise, physical trauma, pregnancy and lactation, high-sugar diet, have also been demonstrated to increase chromium loss (Braunschweig, 1998).

5. Dietary Sources

Processed meats, whole grain products including some ready-to-eat bran cereals, and spices are the best sources of chromium. Dairy products, fruits and vegetables contain low amounts of chromium (Milne, 1994; Nielsen, 1994).

6. Requirements and Recommended Intakes

The current Estimated Adequate and Safe Daily Dietary Intakes for chromium recommended by the National Research Council of the United States are present in Appendix A, Table 11.

7. Chromium Deficiency

Rare chromium deficiency syndromes are reported in the literature, although this may reflect an inability to measure the actual biological activity of this mineral (Herr, 1994). Signs of chromium deficiency found in three women who were received long-term TPN containing low amounts of chromium (Brown et al., 1986; Freund, Atamian, and Fischer, 1979; Jeejeebhoy et al., 1977). One subject exhibited

impaired glucose tolerance and glucose use, weight loss, neuropathy, elevated plasma free fatty acid concentrations, depressed respiratory exchange ratio, and abnormalities in nitrogen metabolism. These abnormalities were alleviated by chromium supplementation. The other subjects developed severe glucose intolerance, weight loss, and a metabolic encephalopathy-like confusional state. All of these abnormalities were reversed by chromium supplementation. In all three cases, however, the chromium-deficient subjects exhibited impaired glucose tolerance, or hyperglycemia with glycosuria, elevated cholesterol, triglyceride, HDL-cholesterol, and a refractoriness to insulin, therefore, these should be considered signs of chromium deficiency (Milne, 1994; Nielsen, 1994).

Causes of chromium deficiency

Factors which increase requirement for chromium or loss of chromium from the body are associated with stressors. These stressors include an elevated intake of simple sugars, strenuous physical exercise or work, infection, and physical trauma (Anderson, 1995; Anderson, 1997; Nielsen, 1994).

8. Chromium Toxicity

Trivalent chromium becomes toxic only at extremely high amounts. Chromium then acts as a gastric irritant rather than as a toxic element interfering with essential metabolism or biochemistry. Industrial exposure to high amounts of chromium, can cause allergic dermatitis, skin ulcers, and bronchogenic carcinoma. Because chromium is a potent sensitizer, external contacts in household or industrial materials can induce an allergic eczema in some people. Chromium toxicity through

oral ingestion, however, is not a practical concern for humans (Milne, 1994; Nielsen, 1994).

9. Assessment of Chromium Status

Chromium concentrations in tissues are 10 to 100 times higher than those in blood. Tissue chromium stores apparently are not in equilibrium with blood chromium stores, therefore, a change in serum chromium concentration is not a good indicator of a mild change in chromium status and not in equilibrium with body stores. However, the relative content of chromium in serum in a chromium-deficient woman maintained on TPN for 3.5 years was markedly lower than those in normal adults. The concentration of serum chromium was also depressed in association with impaired glucose tolerance during acute infection. The serum chromium value may also be an indicator of excessive exposure to chromium. Other indices of chromium status include hair chromium and urinary chromium (Milne, 1994; Nielsen, 1994). The commonly used techniques for determining chromium in biological specimens is atomic absorption spectrophotometry (AAS), recommended as the most practical for clinical and medical research laboratories (Milne, 1994).

The specific biochemical function of chromium has not been identified, therefore, the determination of the amount or activity of some substance directly involving chromium cannot be ascertained and there is no specific biochemical measure of chromium status. An abnormal result of a glucose tolerance test can indicate a low chromium status, and improvement in glucose tolerance after chromium supplementation may be a valid indicator of chromium deficiency (Milne, 1994; Nielsen, 1994).

Cancer

In the United States, malignancy accounted for 526,000 deaths in 1992. Half of the deaths were due to the three most common types of cancer; lung, breast, and colon-rectum. Lung cancer is more prevalent in males, while breast cancer is the most common form of malignancy in females. Cancer of the colon and rectum is equally common in males and females (Mendelsohn, 1994). In Thailand, a review of all new cancer patients registered at Siriraj Cancer Institute, between 1976 and 1995 revealed that ten common cancers in males were lung, liver, nasopharynx, larynx, urinary bladder, esophagus, skin, tongue, lymphoma, and stomach. While those in females were cervix, breast, ovary, skin, thyroid, corpus uteri, lung, liver, mouth, and nasopharynx (Kulwantip Neovakul, 1996).

Cancer typically presents as an abnormal growth which causes illness by production of biochemically active molecules, by local expansion, or by invasion into adjacent or distant tissue sites. The symptoms of the illness depend upon the specific molecular products and the locations of the tumor (Mendelsohn, 1994).

1. The Cell Cycle

All somatic cells, whether normal or malignant, multiply by cell division through the mitotic cell cycle (Figure 3). The cell cycle is marked by two observable events; during S-phase (for synthesis) DNA replication occurs, and during M-phase (for mitosis) cellular division into two daughter cells occurs. G1 (for gap) is the time between the end of mitosis and the start of the next S-phase; G2 is the time between the completion of S-phase and the start of M-phase. Cells that have ceased to proliferate for prolonged periods of time have entered the G0 phase of the cell

cycle (Mendelsohn, 1994). Extracellular factors which influence these processes include growth factors, mitogens and antimitogens, differentiation inducers, cell-cell contact, and nutrients. Mitogens can drive quiescence cells into the cell cycle and antimitogenic signals can drive cells into a quiescence phase. Once a cell passes the restriction point (R), it is committed to progress through S-phase (Kastan, 1997).

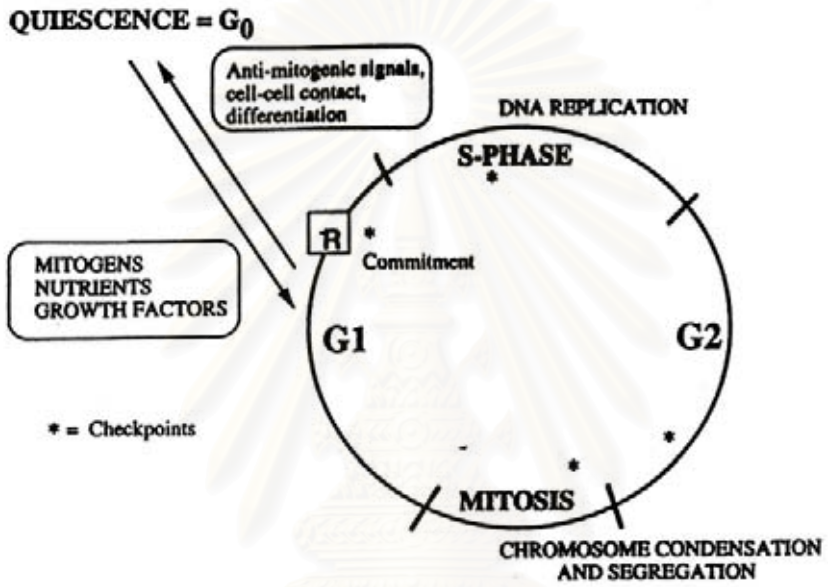

Figure 3. Schematic representation of the cell cycle phases. (Kastan, 1997)

2. Cancer Development

Cancer cells frequently have a diverse set of phenotypic abnormalities, including loss of differentiation, increased motility or invasiveness, that is dysregulation of cell cycle control. Cancer cells replicate themselves faster than normal cells replicate (Kastan, 1997). Tumorigenesis or carcinogenesis is thought to be a multistage process that proceeds on a continuum but is often described in three progressive phases, involving initiation, promotion, and tumor progression. Initiation involves a transformation of the cell produced by the interaction of chemicals,

radiation, or viruses with cellular DNA. The transformation occurs rapidly, but the resultant cell remains dormant for a variable period until activated by a promoting agent. During promotion, initiated cells multiply to form a discrete tumor. From there, progression proceeds. Leading eventually to a fully malignant phenotype with the capacity for tissue invasion and metastasis (Frankmann, 1996).

3. Cancer Therapy

The mainstay of cancer therapy is distributed among three options(Mendelsohn, 1994).

3.1 Surgical therapy

It is still the only curative therapy in many of the most common solid tumors. Surgery has a primary role in the diagnosis, staging and treatment of many tumors.

3.2 Radiation therapy

Its use depends to a large extent on the inherent radiosensitivity of the tumor and the adjacent normal tissues. Ideally radiation therapy should destroy cancerous tissue while causing minimal disruption to surrounding normal structures.

3.3 Chemotherapy

Systemic chemotherapy is the primary treatment available for disseminated malignant disease. Chemotherapy has a significant role in palliation, often with improved survival, in a variety of other tumors.

4. Nutritional Effects of Cancer

The adverse nutritional effects of cancer can be severe and may be compounded by the effects of therapeutic regimens and psychological impact of cancer (Frankmann, 1996). Cancer cachexia is the most common devasting symptoms encountered by cancer patients (McDonald, 2000). The cachexia syndrome is characterized by central nervous system changes in appetite, anorexia, progressive weight loss, asthenia, anemia, evidence of increased cytokine production, abnormalities in carbohydrate, lipid, and protein metabolism, and a change in protein balance, with an increase in "acute phase inflammatory" proteins by the liver, a decrease in protein synthesis in muscle, and increased proteolysis of existing muscle tissue (Baracos, 2000; Frankmann, 1996; McDonald, 2000; Tisdale, 2000). These result in the loss of fat mass, body protein, and decreased serum albumin (Plata-Salaman, 2000).

During cachexia, the organism is maintained in a constant negative energy balance. Cachexia may result not only from hypogeusia, nausea, vomiting, anorexia and a decreased caloric intake but also from malfunction of the gastrointestinal system, losses from the body, cytokine action, and production of substances by tumor cells (Plata-Salaman, 2000). Cytokines are proposed to participate in the development and/or progression of cachexia; interleukin-1, interleukin-6, interferon-γ, tumor necrosis factor, and brain-derived neurotrophic factor have been associated with various cachectic conditions (Frankmann 1996; Plata-Salaman, 2000). Severe imbalances in fluid and electrolyte status may be present in patients with cancer that promote excessive diarrhea or vomiting. Persistent vomiting is associated with intestinal obstruction or intracranial tumors.

The activities of several enzyme systems are affected. Host immunologic function is impaired, apparently as the result of both the neoplasm and the progressive malnutrition (Frankmann, 1996).

Trace Elements and Cancer

There are many reasons to assume that the presence of a malignant neoplasm may produce alterations in the micronutrients of the cancer patients. The rapid, uncontrolled growth of malignant tissue produces a physiologic stress that may vary depending on the tumor. In both animal model systems and human studies, alterations in the acquisition and utilization of nutrients by neoplastic tissue have been observed. For many years, it has been known that the metabolism of several micronutrients is altered in the presence of malignancy (Hoffman, 1985).

Recognition of essential role of many trace elements in a number of biologic processes has led to the assumption that these trace elements may play an important role in the carcinogenic process (Gupta et al., 1993). Both serum zinc and copper are affected by a variety of factors that are not uncommon in cancer patients, i.e., fever, infection, and acute stress. Patients with cancer, especially those with advanced stages of disease, also often demonstrate some degree of nutritional impairment, i.e., weight loss, anorexia, depressed appetite, and/or change in eating patterns that can, if prolonged, lead to trace element deficiencies (Garofalo et al., 1980).

1. Zinc and Cancer

Zinc has been recognized to play an important role as cofactors of superoxide dismutase. This enzyme protects cells against free radical producing agents and substances that might be involved in initiating the neoplastic cells. Zinc is involved in wound healing and possibly in the repair of cellular damage that may be caused by carcinogens (Westin et al., 1989). Zinc is therefore supposed to act as a cellular growth protector, including growth of neoplastic cells. The role of zinc in RNA and DNA polymerase, its inhibitory effects on phosphodiesterase, and its activating effect on membrane-bound adenyl cyclase also suggest a role of zinc in oncogenesis (Poo et al., 1997). Inutsuka and Araki (1978) and Poo et al. found that serum zinc in patients with digestive cancer was significantly lower than those in controls and such patients presented body weight reduction. Those with other types of cancer were similarly observed (C. Pramoolsinsap et al., 1994; Davies et al., 1968; Diez et al., 1989; Gupta et al., 1993; Issell et al., 1981; Lightman et al., 1986; Mellow et al., 1983; Stefanini, 1999; Vikua Skulchan, 1987; Westin et al., 1989). These low serum zinc may be the result of malnutrition related to the neoplastic process, or a decreased albumin concentration which combines to plasma zinc and serves as a plasma zinc carrier (Poo et al., 1997), or the metabolic requirements of cancer cells for zinc results in an increased uptake from the serum (Issell et al., 1981).

The urinary zinc excretion was found to be higher in the colorectal cancer and other types of cancer compared with controls (Hronek et al., 2000; Melichar et al., 1995). The explanation is that zinc has an important antioxidant activity, and a rise in urinary zinc may provide a mechanism of protecting the kidneys from free-radical-induced damage (Hronek et al., 2000).

2. Copper and Cancer

Copper is present in many enzymes involved in oxidation (tyrosinase, amine oxidase, cytochrome c oxidase). Abnormal copper status associated with malignant disease has been known for many years. Many studies have reported a higher serum copper concentration in patients with malignant tumors of digestive organs, colorectal cancer, hepatocellular carcinoma, lung cancer, ovarian malignancy, and breast cancer than those in controls (C. Pramoolsinsap et al., 1994; Diez et al., 1989; Garofalo et al., 1980; Gupta et al., 1993; Inutsuka and Araki, 1978; Lightman et al., 1986; Miatto et al., 1985; Poo et al., 1997).

The elevated serum copper seen in hepatocellular carcinoma patients may be directly related to impaired biliary excretion (Miatto et al., 1985). As the liver is the principal site of copper storage and excretion, as well as the organ in which the plasma copper protein, ceruloplasmin, is synthesized, it may be expected that abnormalities of copper metabolism would be observed in diseases in which liver function is compromised (C. Pramoolsinsap et al., 1994).

Elevations in serum copper levels, however, are most likely due to elevations in ceruloplasmin, which acts as an acute phase reactant (Hoffman, 1985). In addition, increased uptake from the gut, diminished excretion and tissue breakdown with consequent release of copper stores have been suggested as the possible causes for increased serum copper levels (Gupta et al., 1993; Poo et al., 1997). However, the copper metabolism in cancer was described (Linder and Hazegh-Azam, 1996; Braunschweig, 1998). Significant changes in copper absorption, transport, metabolism, or excretion occurred in cancer. In these conditions, serum copper and ceruloplasmin concentrations rose and the rates of synthesis and secretion of ceruloplasmin by the liver were enhanced. At least in the case of inflammation, this occurred through enhanced transcription of ceruloplasmin mRNA in hepatocytes. The increased concentrations of this protein in the circulation most likely provide additional extracellular protection from oxygen radicals to cell membranes during activation of leukocytes, macrophages, and other immune cells that release them. The elevated ceruloplasmin concentrations in cancer would provide additional copper for uptake by cells in normal tissues and perhaps also for abnormal (cancer) cells for synthesis of cuproenzymes or to inactivate the superoxide and other radicals that were produced in areas of inflammation. Copper also plays some roles in angiogenesis, which is required for development of new tissue as well as tumor growth, possibly through the mediation of copper-dependent amine oxidases. Cancer cells readily take up copper from ceruloplasmin, and that, in general, tumor cells contain relatively high concentrations of copper. Other aspects of copper metabolism in the host were also altered in cancer, including enhanced intestinal absorption and diminished turnover of whole-body copper. Decreased retention of copper in intestinal mucosa and liver was

also observed. Not only ceruloplasmin but also other copper-binding components (such as transcuprein) appear to be increased in cancer; the degree of elevation of ceruloplasmin is positively related to disease stage. Assays of ceruloplasmin can therefore aid in the diagnosis of cancer and in assessing disease prognosis and the effectiveness of therapy (Braunschweig, 1998; Linder and Hazegh-Azam, 1996).

3. Chromium and Cancer

El-Yazigi et al. (1988) demonstrated the significantly depleted concentration of chromium in cerebrospinal fluid (CSF) of patients with malignant brain tumors. Apart from the association of chromium with cancer, a precise role for chromium in carcinogenesis has not been defined and there is no direct evidence links it to malignant brain tumors. However, the observed depletion of chromium from CSF may conceivably be attributable to a disturbed metabolism involving chromium in the malignant brain cells. Alternatively, this decrease in CSF concentration may be due to its transfer into the malignant cells as a consequence of a change in the integrity of the membrane.

Trace Elements and Surgery

The acute-phase response to injury, a non-specific response to the stimulus of tissue injury, is a sequence of events involving systemic physiological and biochemical alterations. The main physiological components include fever, an increase in metabolic rate, and leukocytosis. The biochemical changes involve increased oxidation of fat and carbohydrate, an increased transfer of amino acids from skeletal muscle to the liver with the synthesis of hepatic acute-phase proteins, and

alterations in trace element metabolism. These changes are directly under the control of cytokine mediators, interleukin-1, interleukin-6, and tumor necrosis factor (Shenkin, 1995).

Interleukin-1 is ultimately responsible for triggering up-regulation of metallothionein, and resultant zinc accumulation in the liver during acute-phase response, by the following mechanism: Macrophages release interleukin-1 in response to injury, stress, or infection. Interleukin-1 stimulates release of adrenocorticotrophic hormone (ACTH), causing adrenal corticosteroid synthesis and release. The corticosteroids act on hepatocytes to up-regulate metallothionein production and to inhibit further release of interleukin-1 from macrophages. Interleukin-1 also stimulates elaboration of interleukin-6 by fibroblasts. Interleukin-6 increases metallothionein production and zinc uptake by hepatocytes. During acute-phase response, synthesis of albumin diminished while metallothionein and α-2-macroglobulin synthesis is enhanced. These shifts in plasma protein synthesis are thought to be mediated by tumor necrosis factor, interleukin-1 and interleukin-6 (Braunschweig, 1998). The long-term effect of interleukin-1 in cases of injury is toward increased body zinc loss through hyperzincuria (Milne, 1994).

The effect of trauma on serum trace elements in patients undergoing, hysterectomy, cholecystectomy, cardiac surgery, and coronary bypass surgery was reported (Antila et al., 1990; Fraser et al., 1989; Myers et al., 1984). The plasma concentration of zinc fall rapidly after the commencement of an operation. The concentration continues to fall until 12 - 24 hours after the beginning of surgery. Thereafter, plasma zinc may return to normal within 4 - 5 days (Fraser et al., 1989). The decrease in zinc is consistent with evidence that zinc is taken up from the blood

by the liver after stimulation by interleukin-1 (Myers et al., 1984). It is well recognized that the trace element binding proteins albumin also fall in concentration during the acute-phase response. Since most plasma zinc circulates bound to albumin, a significant fall in albumin leads to a fall in zinc concentration (Shenkin, 1995). The mechanism for these responses appears to be primarily through the induction of the metallothionein. The benefits of these changes in plasma zinc are not entirely clear, but one possible advantage would be to increase the availability of zinc in tissues in which metallothionein is induced. Another benefit of the changes in metallothionein is that metallothionein acts as a buffer in providing zinc when required for cell activities, e.g., increased metalloenzyme activity in the pathways of protein synthesis, which are stimulated during the acute-phase response (Shenkin, 1995). Lower serum zinc values after operation seem to suggest as a consequence of high blood losses, disturbances in intracellular zinc metabolism and increased demand for zinc during the anabolic phase (Antila et al., 1990).

Antila et al. (1990), Fraser et al. (1989), and Myers et al. (1984) also reported that major surgery, such as cholecystectomy, cardiac surgery, coronary bypass surgery and hysterectomy, were followed by decreasing in serum copper. Serum copper fell after operation and began to rise after 1 - 2 days, reaching a peak concentration several days after injury. The drop in serum copper was due to a fall in ceruloplasmin, which is a slowly reacting acute-phase protein, caused by an increase in transcapillary escape rate (Myers et al., 1984). The rise in serum copper within 4 - 5 days was probably due to increased synthesis of ceruloplasmin from the liver, which is known to increase after operative trauma (Antila et al, 1990; Myers, 1984).

Ceruloplasmin synthesis is increased by interleukin-1 and also by interleukin-6 (Antila et al, 1990; Shenkin, 1995).

During operation, blood volume is subject to change because of relatively high blood losses, using of crystalloids and plasma expanders, and priming of the heart-lung machine. These result in hemodilution with a corresponding decrease in plasma protein concentrations. Copper is present in plasma bound to carrier protein, and thus its concentration is affected (Antila et al., 1990).

CHAPTER III

MATERIALS AND METHODS

The research protocol and informed consent form were approved by the Ethical Clearance Committee on Human Rights related to Research involving Human Subjects, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok (Appendix B).

Materials

1. Subjects

1.1 Normal subjects

The participants were 60 healthy blood donor subjects (30 men and 30 women) who attended at the National Blood Center, Thai Red Cross Society, Bangkok in August 2000. The following criteria were considered for selection of healthy subjects.

Inclusion criteria

1. Healthy
2. Age 17 - 60 years old
3. Had no previous disease or medical history
4. Nonsmoking and non-alcoholics

5. Have not donated the blood within 3 months before entering the study

Exclusion criteria

1. Taking zinc, copper or chromium supplementations
2. Taking vitamin, mineral supplementations or supplementary foods
3. Taking medication which influences on zinc, copper and chromium levels such as Corticosteroids, Penicillamine, Estrogens and Oral Contraceptives
4. Vegetarians or had poor dietary habits
5. Women in the lactational, menstrual period and pregnant

A short questionnaire was discussed with all subjects. The questionnaire is shown in Appendix C.

1.2 Patients with cancer

In-patients with carcinoma of the gastrointestinal tract admitted at surgical wards, 72nd-year Building, Siriraj Hospital, Bangkok, between September and December 2000, were included in this study. In selection the paitents, the following criteria were considered.

Inclusion criteria

1. In-patients diagnosed as carcinoma of gastrointestinal tract and admitted at surgical wards, 72nd-year Building, Siriraj Hospital
2. Age 15 - 80 years old

3. Underwent operation therapy

Exclusion criteria

1. Had gastrointestinal fistula before operation

2. Taking zinc, copper or chromium supplementations

3. Taking medication which influences on zinc, copper and chromium levels such as Corticosteroids, Penicillamine, Estrogens and Oral Contraceptives

The study design, goals and procedures were discussed with all patients and their legal guardians and signed informed consent agreement were obtained. The consent form is shown in Appendix D.

2. Recording Forms (A copy of each form is present in Appendix E)

2.1 Patient Information Form

2.2 Medication Form

2.3 Serum Zinc, Copper and Chromium Concentration Recording Form

3. Equipments

3.1 Automatic High Speed Refrigerated Centrifuge (Model CR 20B2, Hitachi®, Japan)

3.2 15-ml Plastic Centrifuge Tubes

3.3 1.5-ml Plastic Microcentrifuge Tubes

3.4 10-ml Disposable Syringes and 20-G Disposable Needles

3.5 100-ml Volumetric Flasks

3.6 Pipette Tips

3.7 Micro-pipetter (Autopipetter)

3.8 Electrical Mixer (Vortex Mixer)

3.9 Ice Box

3.10 Oven 60° C

3.11 Deep Freezer –20° C

3.12 Graphite Furnance Atomic Absorption Spectrophotometer (Model Z-8200 Polarized Zeeman, Hitachi®, Japan)

4. Reagents

4.1 Stock Standard Zinc Solution 1000 mg/l (Merck Co., Germany)

4.2 Stock Standard Copper Solution 1000 mg/l (Merck Co., Germany)

4.3 Stock Standard Chromium Solution 1000 mg/l (Merck Co., Germany)

4.4 Concentrated Nitric Acid, AR Grade (Merck Co., Germany)

4.5 Seronorm® Trace Element, Serum (a certified reference serum, Sero Co., Norway)

4.6 Deionized Water

4.7 Ultrapure Water (produced by passing through the Milli-Q Water Purification System for high quality water)

Methods

1. Patient Data Collection

The medical charts of selected patients were reviewed. The data were recorded in Patient Information Form (Appendix E) which was designed and used for recording all necessary information as follows:

1.1 Demographic data: name, hospital number (H.N.), sex, age, weight, height, marital status, address, and occupation

1.2 Diagnosis, and underlying disease

1.3 Operation, and pathologic section

2. Blood Collection

2.1 Normal subjects

Ten milliliters of venous blood was drawn from each subject by using 10-ml disposable syringes and 20-G disposable needles. Blood samples were placed in 15-ml acid-washed plastic centrifuge tubes.

2.2 Patients with cancer

Ten milliliters of venous blood was drawn from each patient in the morning during 6.00 - 8.00 AM on the day before operation (Day 0) and the first and the seventh day after operation (Day 1 and Day 7). Blood samples were placed in 15-ml acid-washed plastic centrifuge tubes.

3. Preparation of Blood Samples

3.1 Normal subjects

Blood samples were allowed to clot at room temperature for 45 minutes in 15-ml acid-washed plastic centrifuge tubes and centrifuged for 10 minutes at the speed of 3,000 rpm to separate serum from whole blood. One milliliter of serum was pipetted into a 1.5-ml plastic microcentrifuge tube. While it was stirred vigorously on a vortex mixer, fifty microliter of concentrated nitric acid was added. Then, after the mixture was heated in a 70° C-water bath for 5 minutes, it was centrifuged for 5 minutes at 1,000 g. The clear supernatant was transferred to another 1.5-ml plastic microcentrifuge tube and stored at –20° C in deep freezer until analysis was performed (Nomoto, 1988; Smith et al., 1979; Young and Bermes, 1994).

3.2 Patients with cancer

Blood samples were prepared in the same procedure as previously described in normal subjects.

4. Preparation of Glassware and Plastic Utensils

Precautions must be taken throughout all sampling, preparation and analytical procedures to avoid zinc, copper and chromium contamination. All glassware and plastic (polyethylene) utensils were thoroughly cleaned with detergent, washed with water then soaked in 20% nitric acid for at least 12 hours (overnight), rinsed with deionized water and ultrapure water, respectively, in order to let them free from any trace element. All were air-dried at 60° C, then kept out of contamination in a dust-free environment (in the tightly closed containers) (Association of Official Analytical Chemists [AOAC], 1995; Milne, 1994; Poo et al., 1997).

5. Analytical Methods

Serum zinc, copper and chromium concentrations were determined by graphite furnance atomic absorption spectrophotometer (Hitachi® Model Z-8200 Polarized Zeeman instrument). Each sample was analyzed in duplicate. In each analytical run, the quality control was accomplished by using aliquots of a Seronorm®, a commercial certified reference serum, as a control (Milne, 1994). The serum zinc, copper, and chromium concentration of samples were determined from the standard curve. Details of the determination of serum zinc, copper, and chromium are described in Appendix F.

6. Classification of Cancer Patients

The cancer patients were categorized into 3 groups according to aggressiveness and prognosis of the disease as shown in Table 4 in order to study the influence of cancer progression on serum zinc, copper, and chromium concentration.

Table 4. Provisional criteria for categorizing cancer patients according to aggressiveness and prognosis of the disease

Nominal category	**Principal characteristics**
Group 1	a) Have no problem of eating and swallowing (dysphagia) b) Relatively less aggressive tumor with reasonable survival (5-year survival is more than 30%)
Group 2	a) Have problems of eating and swallowing (dysphagia) due to obstruction of gastrointestinal tract b) Relatively aggressive tumor with poor survival (less than 1-year survival, and cancer cachexia is common)
Group 3	a) Have no problem of eating and swallowing (dysphagia) b) Relatively aggressive tumor with poor survival (less than 1-year survival, and cancer cachexia is common)

In order to study the influence of surgery on serum zinc, copper, and chromium concentration, the cancer patients were categorized into 3 groups according to the extent of surgery as shown in Table 5.

Table 5. Provisional criteria for categorizing cancer patients according to the extent of surgery

Nominal category	**Surgical procedure**
Group 1	Relatively minor operative procedure a) Operative time is less than 1 hour b) No blood loss
Group 2	Major operation a) Operative time is 1 – 2 hours
Group 3	Major operation a) Operative time is more than 2 hours b) Have extensive organ dissection and resection

7. Statistical Analysis

(1) To determine the effect of cancer disease condition, mean differences of serum zinc, copper, and chromium concentration of cancer patients in each disease group were compared with normal subjects by t-test (Gardiner, 1997).

(2) To determine the effect of surgery, means differences of serum zinc, copper, and chromium concentration of cancer patients in each surgery group on the day before and after operation (Day 0 VS Day 1, and Day 0 VS Day 7) were compared by paired t-test with SPSS program for windows version 9.0 (Gardiner, 1997).

CHAPTER IV

RESULTS

Blood specimens in this study were obtained from 60 healthy normal subjects and 44 surgical patients with gastrointestinal cancer. The demographic data of age, sex, and height is shown in Table 6. The distribution of demographic data between normal subjects and cancer patients is comparable.

Table 6. Demography of normal subjects and cancer patients

Character	Normal subjects (N = 60)	Cancer patients (N = 44)
Age (years)	33.48 ± 9.87	57.48 ± 10.44
Sex (Male : Female)	1 : 1	1.44 : 1
Height (cm)	164.62 ± 7.99	158.16 ± 9.47

1. Serum zinc, copper, and chromium concentration of normal subjects

Data on age, weight, height, body mass index (BMI), serum zinc, copper, and chromium concentration of 60 normal subjects (30 men and 30 women) are shown in Appendix G, Table 15.

2. Serum zinc, copper, and chromium concentration of cancer patients

Forty-four in-patients with gastrointestinal cancer (26 men and 18 women) were included in this study. The clinical characteristics, surgical treatment, and serum zinc, copper, and chromium concentration of cancer patients are present in Appendix G, Tables 16 and 17.

The means serum zinc, copper, and chromium concentration between normal subjects and the entire population of cancer patients are present in Table 7. These data are also present graphically in Appendix H, Figure 7.

The effects of cancer progression and the extent of surgery on serum zinc, copper, and chromium concentration were determined. The cancer patients were categorized according to the criteria as previously shown in Tables 4 and 5. The number of cancer patients categorized by aggressiveness and prognosis of the disease is present Appendix G, Table 18. Group 1, 2, and 3 consisted of 19, 12, and 13 patients, respectively. The number of cancer patients categorized by the extent of surgery is presented in Appendix G, Table 19. Group 1, 2, and 3 consisted of 11, 21, and 12 patients, respectively.

The means serum zinc, copper, and chromium concentration of cancer patients categorized by aggressiveness and prognosis of the disease and normal subjects are present in Table 8. These data are also present graphically in Appendix H, Figure 8.

Table 7. Means serum zinc, copper, and chromium concentration between normal subjects and the entire population of cancer patients*

Group	Serum zinc (μg/dl)	Serum copper (μg/dl)	Serum chromium (μg/l)
Normal subjects (N = 60)	84.58 ± 16.21	89.08 ± 16.94	0.47 ± 0.15
Cancer Patients (N = 44)			
Day 0 [1]	86.58 ± 46.97	152.95 ± 51.81 [a]	0.26 ± 0.14 [a]
Day 1 [2]	62.70 ± 52.46 [a, b]	128.35 ± 44.68 [a, b]	0.14 ± 0.08 [a, b]
Day 7 [3]	82.70 ± 62.49	144.38 ± 42.60 [a, b]	0.21 ± 0.10 [a, b]

* Value expressed as mean ± SD

[1] Collected blood samples on the day before operation

[2] Collected blood samples on the first day after operation

[3] Collected blood samples on the seventh day after operation

[a] Significant difference at $p < 0.05$ (compared with normal subjects)

[b] Significant difference at $p < 0.05$ (compared with Day 0)

Table 8. Means serum zinc, copper, and chromium concentration of normal subjects and cancer patients categorized by aggressiveness and prognosis of the disease*

Group	No. of subjects	Serum zinc (μg/dl)	Serum copper (μg/dl)	Serum chromium (μg/l)
Normal	60	84.58 ± 16.21	89.08 ± 16.94	0.47 ± 0.15
Group 1 [1]	19	94.16 ± 53.12 [a]	127.63 ± 31.35 [**, a]	0.29 ± 0.14 [**, a]
Group 2 [2]	12	95.92 ± 37.64 [a]	137.08 ± 38.70 [**, a]	0.23 ± 0.15 [**, a]
Group 3 [3]	13	66.88 ± 42.13 [b]	204.62 ± 51.10 [**, b]	0.25 ± 0.12 [**, a]

* Value expressed as mean ± SD (obtained from Day 0)

[1] Patients who have no associated disphagia, relatively less aggressive tumor with reasonable survival

[2] Patients who have associated disphagia, relatively aggressive tumor with poor survival

[3] Patients who have no associated disphagia, relatively aggressive tumor with poor survival

** Significant difference at $p < 0.05$ (compared with normal subjects)

The different letters (a, b) show a significant difference at $p < 0.05$

The means serum zinc, copper, and chromium concentration of cancer patients categorized by the extent of surgery and normal subjects are present in Table 9. These data are also present graphically in Appendix H, Figure 9A, 9B, and 9C.

Table 9. Means serum zinc, copper, and chromium concentration of normal subjects and cancer patients categorized by the extent of surgery*

Serum trace elements	Normal subjects (N = 60)	Group [4] (N = 11)	Group 2 [5] (N = 21)	Group 3 [6] (N = 12)
Serum zinc (μg/dl)	84.58 ± 16.21			
Day 0 [1]		103.18 ± 33.47 [a]	69.50 ± 47.40	101.25 ± 49.51
Day 1 [2]		65.45 ± 38.55 [b]	64.95 ± 60.51	56.25 ± 51.84 [a, b]
Day 7 [3]		87.95 ± 51.29	81.86 ± 75.00	79.38 ± 51.03 [b]
Serum copper (μg/dl)	89.08 ± 16.94			
Day 0 [1]		153.18 ± 30.48 [a]	167.86 ± 55.04 [a]	126.67 ± 54.41 [a]
Day 1 [2]		142.95 ± 33.70 [a, b]	140.95 ± 45.25 [a, b]	92.92 ± 34.11 [b]
Day 7 [3]		147.05 ± 31.16 [a]	154.05 ± 49.00 [a, b]	125.00 ± 35.37 [a]
Serum chromium (μg/l)	0.47 ± 0.15			
Day 0 [1]		0.25 ± 0.18 [a]	0.27 ± 0.11 [a]	0.26 ± 0.14 [a]
Day 1 [2]		0.11 ± 0.05 [a, b]	0.16 ± 0.09 [a, b]	0.14 ± 0.08 [a, b]
Day 7 [3]		0.16 ± 0.08 [a]	0.23 ± 0.11 [a]	0.20 ± 0.09 [a]

* Value expressed as mean ± SD

[1] Collected blood samples on the day before operation

[2] Collected blood samples on the first day after operation

[3] Collected blood samples on the seventh day after operation

[4] Patients who received relatively minor operative procedure (operative time < 1 hr)

[5] Patients who received major operation (operative time 1-2 hr)

[6] Patients who received major operation (operative time > 2 hr, have extensive organ dissection and resection)

[a] Significant difference at $p < 0.05$ (compared with normal subjects)

[b] Significant difference at $p < 0.05$ (compared with day 0)

CHAPTER V

DISCUSSION

The presence of a malignant neoplasm may produce alterations in the micronutrients status of the cancer patients. The rapid, uncontrolled growth of malignant tissue produces a physiologic stress that may vary depending on the tumor. In both animal model systems and human studies, alterations in the acquisition and utilization of nutrients by neoplastic tissue have been observed. For many years, it has been known that the metabolism of several micronutrients is altered in the presence of malignancy (Hoffman, 1985). Trace elements may play an important role in the carcinogenic process (Gupta et al., 1993). This study was undertaken to determine the effects of cancer disease on serum zinc, copper, and chromium concentration.

Serum zinc, copper, and chromium concentrations were determined in 60 normal subjects and 44 surgical patients with gastrointestinal cancer by atomic absorption spectrophotometry.

1. Serum zinc, copper, and chromium concentration of normal subjects

The serum zinc concentration in normal subjects found in this study is in the range of 55 - 130 μg/dl. This result is within the range reported by Jongkolnee Pimpton (1996) (73.72 - 117.31 μg/dl), determined in 100 healthy blood donors at Bhumibol Adulyadej Hospital. Serum copper concentration (60 - 140 μg/dl) is within the range studied by Jongkolnee Pimpton (82.12 - 136.42 μg/dl) as well. This may be

due to the same geographical and environmental factors, the same diet and racial influences. Serum chromium concentration (0.19 - 0.93 μg/l) is comparable with that reported for healthy adults as a reference intervals (0.12 - 2.10 μg/l) (Milne, 1994).

The accepted reference interval for zinc in serum is 70 - 150 μg/dl, for serum copper is 80 - 155 μg/dl in women and 70 - 140 μg/dl in men, and for serum chromium concentration is 0.12 - 2.10 μg/l (Milne, 1994). In this study, serum zinc, copper, and chromium concentrations are comparable with these reference intervals.

2. Serum zinc, copper, and chromium concentration of cancer patients

2.1 Serum zinc, copper, and chromium concentration of cancer patients categorized by aggressiveness and prognosis of the disease

Collectively, the average serum zinc concentration in the entire studied population of cancer patients in this study is not significantly different from normal subjects. However, when the cancer patients were categorized into 3 different groups by aggressiveness and prognosis of the disease, the mean serum zinc concentration of patients in group 3 (hepatocellular carcinoma, cholangiocarcinoma, cancer head of pancreas, and cancer ampulla of vater) is significantly lower than group 1 and group 2 (Table 8 and Figure 8). C. Pramoolsinsap et al. (1994) also reported the decrease of serum zinc in patients with hepatocellular carcinoma. Inutsuka and Araki (1978) and Poo et al. (1997) found that serum zinc in patients with digestive cancer was significantly lower than those in controls. Those with other types of cancer were similarly observed (Davies et al., 1968; Diez et al., 1989; Gupta et al., 1993; Issell et al., 1981; Lightman et al., 1986; Mellow et al., 1983; Stefanini, 1999; Vikua Skulchan, 1987; Westin et al., 1989). The low serum zinc may be the result of

malnutrition related to the neoplastic porcess, or a decreased albumin concentration which combines to plasma zinc and serves as a plasma zinc carrier (Poo et al., 1997), or the metabolic requirements of cancer cells for zinc results in an increased uptake from the serum (Issell et al., 1981).

Patients in group 3 of this study had no associated obstruction (or dysphagia) but the serum zinc level is lower than that in group 2 which had problems of eating and swallowing due to obstruction of gastrointestinal tract. This may be due to patients with relatively aggressive tumor with poor survival (group 3) have more aggressive nature of disease and more profound cancer cachexia resulted from hypermetabolism and cytokines production (i.e., interleukin-1, interleukin-6, and tumor necrosis factor) (Plata-Salaman, 2000).

The decrease in mean concentration of serum zinc in cancer patients may also be due to a trap consuming zinc of tumor tissue, possibly for cell growth (Issell et al., 1981; Westin et al., 1989) or malnutrition related to the neoplastic process as reported in patients with breast cancer (Garofalo et al., 1980).

Serum zinc level declines found in this study indicated that the patients are at risk of developing clinical zinc deficiency. Thus, supplementation of zinc for cancer patients especially patients of group 3 appears to be warranted at the time of admission.

In the present study, the mean serum copper concentration of gastrointestinal cancer patients in group 1, 2, and 3 are significantly higher than those of normal subjects. The highest serum copper concentration was found in patients of group 3 (Table 8 and Figure 8) and significantly higher than group 1 and group 2.

These results are in agreement with the previous studies in patients with digestive (Inutsuka and Araki, 1998; Poo et al., 1997), colorectal (Gupta et al., 1993), hepatic (C. Pramoolsinsap et al., 1994; Miatto et al., 1985), breast (Garofalo et al., 1980), ovary (Lightman et al., 1986) and lung cancer (Diez et al., 1989).

Serum copper concentration of cancer patients in group 3 is significantly higher than those in group 1 and group 2. Patients in group 3 had no associated obstruction (or dysphagia) but the serum copper level is higher than that in group 2 which had problems of eating and swallowing due to obstruction of gastrointestinal tract. This again, may be due to the more aggressive nature of the disease and the effect of cytokines and hypermetabolism in these patients, despite absence to dysphagia.

The mechanism for the elevated level of serum copper is still unclear. Increased ceruloplasmin levels (Fischer and Shifrine, 1978), increased uptake from the gut, diminished excretion and tissue breakdown with consequent release of copper stores have been suggested as the possible cause for increased serum copper concentrations (Gupta et al., 1993; Poo et al., 1997).

In patients with hepatoma, diffuse process like cirrhosis may affect copper metabolism. C. Pramoolsinsap et al. (1994) had reported the abnormalities of copper metabolism in patients whose liver function was impaired because the liver is the principal site of copper storage and excretion, as well as the synthesis of serum copper protein, ceruloplasmin.

In cancer, serum copper and ceruloplasmin concentration rise and the rate of synthesis and secretion of ceruloplasmin by the liver are enhanced. The elevated ceruloplasmin concentrations in cancer would provide additional copper for uptake by cells in normal tissues and perhaps also for abnormal (cancer) cells. Copper is also required for development of new tissue as well as tumor growth (Linder and Hazegh-Azam, 1996). Not only ceruloplasmin but also other copper-binding components (such as transcuprein) appear to be increased in cancer; the degree of elevation of ceruloplamin is positively related to disease stage (Braunschweig, 1998; Linder and Hazegh-Azam, 1996).

Serum copper level is elevated in all groups even in patients with aggressive cancer. Gupta et al. (1993) also found that copper level in cancerous colorectal tissue was increased compared to non-cancerous colorectal tissue. Copper replacement may not be needed or even have to consider deletion of this trace element from replacement fluid formula.

Information of chromium in health and disease especially in cancer is exceedingly scarce. Most of the chromium values previously reported were acquired from workers with industrial exposure to high amount of chromium (Milne, 1994; Nielson, 1994). In this study, the mean concentration of chromium in serum of cancer patients in group 1, 2, and 3 are significantly lower than those in normal subjects (Table 8 and Figure 8). El-Yazigi et al. (1988) also found a significant depleted chromium in cerebrospinal fluid of patients with malignant brain tumors.

Apart from the association of chromium with cancer, a precise role for chromium in cancerigenesis has not been defined. However, the observed depletion of chromium level may conceivably be attributable to a disturbed metabolism involving chromium in the malignant cells (El-Yazigi et al., 1988). The decrease in serum chromium concentration may also be due to malnutrition related to neoplastic process (Garofalo et al., 1980) or cancer cachexia which resulted from malfunction of the gastrointestinal system, hypermetabolism and cytokines production (i.e., interleukin-1, interleukin-6, and tumor necrosis factor) (Plata-Salaman, 2000).

The consistent finding of low serum chromium level in all groups of cancer patients indicated that clinical chromium deficiency is prone. Thus, chromium replacement might be warranted.

2.2 Serum zinc, copper, and chromium concentration of cancer patients categorized by the extent of surgery

A significant decrease in mean serum zinc concentration on the first day after operation (Day 1) in patients of group 1 and 3 is observed, compared with that on the day before operation (Day 0) ($p < 0.05$) (Table 9 and Figure 9A). However, after 7 days of operation, the serum zinc concentration is increased and closed to the value of that in the day before operation. Antila et al. (1990), Fraser et al. (1989), and Myers et al. (1984) have reported the decrease in serum zinc concentration in patients undergoing coronary bypass operation, cholecytectomy, cardiac surgery, and hysterectomy and serum zinc returned to normal within 4-5 days.

The degree of decrease in serum zinc concentration tends to parallel with the extent of surgery. The maximal fall of serum zinc concentration is remarkable observed in patients of group 3 (56.25 ± 51.84 μg/dl) which received the most extensive operation (abdomino-peritoneal resection, gastrectomy, spleenectomy, tumor resection with hepaticojejunostomy, and pancreaticoduodenectomy).

The decrease in mean serum zinc concentration may be related to a redistribution of zinc to the site of tissue injury and zinc can also decrease in serum as a non-specific reaction to stress (Fraser et al., 1989). The non-specific response to the stimulus of tissue injury is a sequence of physiological and biochemical alterations include fever, increase in metabolic rate and leukocytosis, increased oxidation of fat and carbohydrate, increased transfer of amino acids from skeletal muscle to the liver with the synthesis of hepatic acute-phase proteins, and alterations in trace element metabolism. These changes are directly under the control of cytokine mediators, interleukin-1, interleukin-6, and tumor necrosis factor (Shenkin, 1995). Zinc is taken up from the blood by the liver after stimulation by interleukin-1 (Myers et al., 1984).

The decrease in serum zinc concentration may also be due to hemodilution, the plasma proteins were diluted by fluid infusion (Antila et al., 1990). Serum zinc is mostly bound to albumin, therefore, the decrease in albumin and serum zinc concentration were seen after operation (Antila et al., 1990; Myers et al., 1984). In this study, the decrease in serum albumin is also observed after operation in 12 patients.

Serum zinc level is depressed in cancer patients. The depression is further aggravated by operation. This finding indicated that zinc replacement should be routinely included in preoperative and postoperative supplementation.

Serum copper concentration is found to be significantly decreased on Day 1 in patients of group 1, 2, and 3 compared with serum copper concentration on Day 0 ($p < 0.05$) (Table 9 and Figure 9B). The serum copper concentration increases gradually during the next 7 days after operation (Day 7). Antila et al. (1990), Fraser et al. (1989), and Myers et al. (1984) have reported the decrease in serum copper concentration in patients undergoing coronary bypass operation, cholecytectomy, cardiac surgery, and hysterectomy. Serum copper fell after operation and began to rise after 1 - 2 days, reaching a peak concentration several days after injury. The drop in serum copper was due to a fall in ceruloplasmin, which is a slowly reacting acute-phase protein, caused by an increase in transcapillary escape rate (Myers et al., 1984). The rise in serum copper within 4 - 5 days was probably due to increased synthesis of ceruloplasmin from the liver, which is known to increase after operative trauma (Antila et al., 1990; Myer, 1984).

The degree of decrease in serum copper concentration tends to parallel with the extent of surgery. The maximal fall of serum copper concentration is most remarkable in patients of group 3 (92.92 ± 34.11 μg/dl).

The decrease in serum copper concentration may be due to hemodilution, the plasma proteins were diluted by fluid infusion (Antila et al., 1990). Furthermore, the drop in serum copper concentration was also due to a fall in ceruloplasmin, an acute-phase protein, caused by an increase in transcapillary escape rate (Myers et al., 1984). The gradually rise in serum copper concentration during the next 7 days after operation was probably due to increased synthesis of the carrier protein, ceruloplasmin, induced by interleukin-1 and also by interleukin-6 (Antila et al., 1990; Myers et al., 1984; Shenkin, 1995).

This study shows the consistent elevation of serum copper in all patient groups, eventhough the serum copper concentration is slightly decreased after operation, it returns to supranormal level within a week. Therefore, copper supplementation is not necessary and it should be deleted from the routine replacement fluid formula to save cost and minimize complication.

Serum chromium concentration on Day 1 in patients of group 1, 2, and 3 is significantly decreased compared with serum chromium concentration on Day 0 ($p < 0.05$) (Table 9 and Figure 9C). The mean serum chromium concentration increases on the 7^{th} day after operation (Day 7).

The decrease in serum chromium concentration may be due to hemodilution, the plasma proteins were diluted by fluid infusion (Antila et al., 1990). Serum chromium is mostly bound to transferrin or albumin, therefore, the decrease in serum transferrin or albumin may lead to the decrease in serum chromium concentration after operation. Factors which increase requirement for chromium or

loss of chromium from the body are associated with stressors include infection and physical trauma (Anderson, 1995; Anderson, 1997; Nielsen, 1994).

Chromium level is depressed in cancer patients. The depression is further aggravated by operation. This finding indicated that chromium replacement should be included in preoperative and postoperative supplementation as a routine.

CHAPTER VI

CONCLUSION

Serum zinc, copper, and chromium concentrations were determined in 60 normal subjects and 44 patients with gastrointestinal cancer admitted at surgical wards of Siriraj Hospital during September - December 2000.

The means serum zinc, copper, and chromium concentration of 60 normal subjects are in the reference intervals.

The mean serum zinc concentration is decreased and lowest in cancer patients of group 3 (hepatocellular carcinoma, cholangiocarcinoma, cancer head of pancreas, and cancer ampulla of vater), which have the most aggressive cancer. The means serum chromium concentration in all groups of cancer patients are significantly lower than those of normal subjects. The means serum zinc and chromium concentration are decreased in cancer patients and further aggravated by operation. This finding indicated that the patients are at risk of developing clinical zinc and chromium deficiency. Therefore, supplementation of zinc and chromium for cancer patients especially patients of group 3 appear to be warranted at the time of admission and after operation.

The means serum copper concentration are elevated in all groups even in patients with aggressive cancer. Eventhough the serum copper concentration is slightly decreased after operation, it returns to supranormal level within a week. Copper supplementation may not be needed or even have to consider deletion of this trace element from replacement fluid formula.

Suggestion for Further Study

Before firmly recommend that copper should be deleted from the routine supplementation, further studies are needed to determine

1. the consistency of the deviation of elevated serum copper concentration in cancer patients
2. whether the elevated serum copper concentration, mostly bound to plasma protein, can function physiologically

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APPENDICES

APPENDIX A

Recommended Dietary Intakes of Zinc, Copper, and Chromium

Table 10. Recommended Dietary Allowances (RDA)*

Category	Age (years) or condition	Zinc (mg)
Infants	0.0 – 0.5	5
	0.5 - 2.0	5
Children	1 – 3	10
	4 – 6	10
	7 – 10	10
Males	11 – 14	15
	15 – 18	15
	19 – 22	15
	23 – 50	15
	51+	15
Females	11 – 14	12
	15 – 18	12
	19 – 22	12
	23 – 50	12
	51+	12
Pregnancy		15
Lactation		+19

* From Food and Nutrition Board, National Research Council: Recommended Dietary Allowances, 10th ed. Washington, D.C., National Academy of Sciences, 1989.

Table 11. Estimated Adequate and Safe Daily Dietary Intakes*

Category	Age (years) or condition	Copper (mg)	Chromium (μg)
Infants	0.0 - 0.5	0.4 - 0.6	10 - 40
Children and adolescents	0.5 - 1.0	0.6 - 0.7	20 - 60
	1 – 3	0.7 - 1.0	20 - 80
	4 – 6	1.0 - 1.5	30 - 120
	7 – 10	1.0 - 2.0	50 - 200
	11+	1.5 - 2.5	50 - 200
Adults		1.5 - 3.0	50 - 200

* From Food and Nutrition Board, National Research Council: Recommended Dietary Allowances, 10th ed. Washington, D.C., National Academy of Sciences, 1989.

APPENDIX B

Documentary Proof of Ethical Clearance Committee

on Human Rights

๒ ถนนพรานนก บางกอกน้อย กรุงเทพฯ ๑๐๗๐๐
โทร. ๔๑๑-๑๔๒๙, ๔๑๑-๓๒๕๓
โทรสาร. ๖๖-๒-๔๑๒-๑๓๗๑

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Faculty of Medicine Siriraj Hospital
Mahidol University

DOCUMENTARY PROOF OF ETHICAL CLEARANCE COMMITTEE
ON HUMAN RIGHTS RELATED TO RESEARCH INVOLVING HUMAN SUBJECTS
FACULTY OF MEDICINE SIRIRAJ HOSPITAL MAHIDOL UNIVERSITY, BANGKOK, THAILAND

TITLE OF PROJECT : SERUM ZINC, COPPER AND CHROMIUM CONCENTRATION IN NORMAL SUBJECTS AND SURGICAL PATIENTS WITH CANCER IN SIRIRAJ HOSPITAL

PRINCIPAL INVESTIGATOR : MISS NUALNIT WICHIEN

NAME OF DEPARTMENT : DEPARTMENT OF PHARMACY

APPROVED BY COMMITTEE ON HUMAN RIGHTS RELATED TO RESEARCH INVOLVING HUMAN SUBJECTS ON 11 SEPTEMBER 2000

SIGNATURE OF CHAIRMAN : .....
PROF. KHUN NANTA MARANETRA
M.D.,Grad Dip Clin Sc Med, Msc Med (BKK), Thai Board Resp Med, Thai Board Crit care Med
MD (Melb.), FRCPT, FCCP, FRACP,FRCP (Lond.) , FRCP (Glasg.) , FRCP (Edin.)

SIGNATURE OF DEAN : .....
PROF. DR. CHANIKA TUCHINDA
M.D., MS, FAAP.

11 SEPTEMBER 2000

APPENDIX C

Questionnaire for Blood Donors Attended at the

Thai Red Cross Society

1. General Information

Name……Last name……..Unit No……

Sex □ Male □ Female

Age……..years Weight .……kg Height……..……cm

Address……

Phone……

2. Have you ever donate blood within the last 3 months? □ Yes □ No

3. Are you in a menstrual period? □ Yes □ No

4. Are you pregnant? □ Yes □ No

5. Are you in a lactational period? □ Yes □ No

6. In general, how would you rate your health? □ Excellent □ Good

□ Fair □ Poor

7. Dietary habits

Do you have an adequate nutrient intake? □ Yes □ No

Have you had a recent weight loss within 3 months? □ Yes □ No

Have you been on a weight reduction diet? □ Yes □ No

Have you had a recent change in appetite? □ Yes □ No

Do you eat vegetarian food? □ Yes □ No

How often?……

Do you drink alcohol? □ Yes □ No

How often?……How much?……Type……..

Do you smoke? □ Yes □ No

How often?……How much?……

8. What disease have you experienced within the last 3 months?

Asthma, Seizure, Tuberculosis, Allergies, Hepatitis, Hypertension, Cardiovascular disease, Diabetes Mellitus, Renal disease, Thyroid disease, Cancer, Other (specify)……

9. What medications are you presently taking within the last 3 months?

□ Penicillamine

□ Diuretics

□ Corticosteroids

□ Other (specify)……

10. What vitamin/mineral supplements are you presently taking within the last 3 months?

□ Multivitamins

□ Zinc supplements (e.g. ZBEC, Stresstab, or others)

□ Other (specify)……

11. What hormones are you presently taking within the last 3 months?

□ Estrogens

□ Oral contraceptives

□ Other (specify)……

APPENDIX D

Consent Form

แบบยินยอมเข้าร่วมการศึกษา

วันที่.....เดือน.....พ.ศ.....

ข้าพเจ้า.....อายุ.....ปี อาศัยอยู่บ้านเลขที่.....ถนน.....

แขวง.....เขต.....จังหวัด.....

ได้ทราบรายละเอียดของโครงการวิจัยเรื่อง ระดับสังกะสี ทองแดง และโครเมียมในซีรัมของคนปกติ และผู้ป่วยศัลยกรรมโรงพยาบาลศิริราช ดังต่อไปนี้

ก. ข้าพเจ้าทราบวัตถุประสงค์ของการวิจัย คือ เพื่อศึกษาผลของสภาวะโรค และการผ่าตัด ต่อปริมาณสังกะสี ทองแดง โครเมียมในซีรัม

ข. ข้าพเจ้าทราบว่า ผู้เข้าร่วมวิจัยจะได้รับการดูแลรักษาตามขั้นตอนที่ถูกต้องไม่แตกต่างจากผู้ป่วยโรคเดียวกันที่ไม่ได้เข้าร่วมการวิจัยแต่อย่างใด

ค. ข้าพเจ้าทราบว่า จะได้รับประโยชน์จากการเข้าร่วมโครงการวิจัยในการตรวจหาปริมาณสังกะสี ทองแดง และโครเมียมในซีรัม โดยไม่เสียค่าใช้จ่าย ผลการตรวจวิเคราะห์จะเก็บไว้ในแฟ้มผู้ป่วยพร้อมที่จะให้แพทย์อ่าน หากมีภาวะการขาดแร่ธาตุดังกล่าว อาจได้รับการรักษา เมื่อแพทย์ผู้รักษาพิจารณาว่าเหมาะสม

ง. ข้าพเจ้าทราบว่า ความเสี่ยงต่างๆที่อาจเกิดขึ้น คือการติดเชื้อจากการแทงเข็มเจาะเลือด แต่โอกาสเกิดน้อยมาก เนื่องจากเข็ม และกระบอกฉีดยาที่ใช้ เป็นการเปิดใช้ใหม่ทันที อย่างไรก็ตาม ผู้เข้าร่วมวิจัยจะได้รับการแก้ไขอย่างรวดเร็ว และจะได้รับการระมัดระวังอย่างเต็มที่มิให้เกิดขึ้น ถ้าบังเอิญเกิดขึ้น จะได้รับการดูแลจนกว่าจะหายเป็นปกติ

หากข้าพเจ้ามีข้อสงสัยประการใดหรือเมื่อเกิดผลข้างเคียงจากการวิจัยขึ้น ข้าพเจ้าจะติดต่อกับ

1. ภญ. นวลนิตย์ วิเชียร ได้ที่ 120/5 หมู่ 9 ซ.ภาณุ 3 หมู่บ้านภาณุรังษี ถ.บางกรวย-ไทรน้อย ต.บางกรวย อ.บางกรวย จ.นนทบุรี 11130 โทร 4221571, pager 1144 เรียก 725590
2. อาจารย์ที่ปรึกษาวิทยานิพนธ์ รศ. ดร. อรอนงค์ กังสดาลอำไพ
   ภาควิชาอาหารเคมี คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย โทรศัพท์ 2188292
3. อาจารย์ที่ปรึกษาวิทยานิพนธ์ร่วม ผศ. นพ. ธัญเดช นิมมานวุฒิพงษ์
   ศูนย์โภชนบำบัด คณะแพทยศาสตร์ศิริราชพยาบาล โทรศัพท์ 4197740

หากข้าพเจ้าได้รับผลข้างเคียงจากการวิจัย ข้าพเจ้าจะได้รับการปฏิบัติ/การชดเชยโดยข้าพเจ้าจะได้รับการดูแลแก้ไขและรักษาอย่างดีที่สุด โดยไม่เสียค่าใช้จ่ายใดๆ

หากผู้วิจัยมีข้อมูลเพิ่มเติมทั้งด้านประโยชน์และโทษที่เกี่ยวข้องกับการวิจัยนี้ ผู้วิจัยจะแจ้งให้ข้าพเจ้าทราบอย่างรวดเร็วโดยไม่ปิดบัง

ข้าพเจ้ามีสิทธิ์ที่จะของดการเข้าร่วมโครงการวิจัยโดยมิต้องแจ้งให้ทราบล่วงหน้าโดยการงดการเข้าร่วมการวิจัยนี้จะไม่มีผลกระทบต่อการได้รับบริการหรือการรักษาที่ข้าพเจ้าจะได้รับแต่ประการใด

ข้าพเจ้าได้รับทราบจากผู้วิจัยว่า จะไม่เปิดเผยข้อมูลหรือผลการวิจัยของข้าพเจ้าเป็นรายบุคคลต่อสาธารณชน

ข้าพเจ้าได้รับทราบและได้ซักถามผู้วิจัยจนหมดข้อสงสัยโดยตลอดแล้วและยินดีเข้าร่วมในการวิจัย จึงได้ลงลายมือชื่อไว้เป็นหลักฐานต่อหน้าพยาน

ลงชื่อ.....ผู้ยินยอมหรือผู้แทนโดยชอบธรรม

(.....)

ลงชื่อ..... หัวหน้าโครงการวิจัย

(นางสาวนวลนิตย์ วิเชียร)

ลงชื่อ.....พยาน

(.....)

ลงชื่อ.....พยาน

(.....)

APPENDIX E

Recording Forms

Patient Information Form

Ward……..Bed……

1. Name……..……..Last Name…….……

2. HN……AN…….……..

3. Sex □ Male □ Female

4. Age……years Weight……kg Height…….cm.

5. Marital status □ Single □ Married . □ Divorced □ Widowed

6. Occupation……

7. Address……..……..

8. Admit date…….……..Discharge date…….……

9. Admit diagnosis……..……

10. Underlying disease……..…….

11. Chief Complaint (CC)……..…….

12. History of present illness (HPI)……

……

……

……

……

……

13. Past medical history (PMH)……

……

……

……

……

……

14. History of allergies……

15. Social history (SH)……

16. Family history (FH)……

17. Physical examination……

……

……

18. Operation……Date…..……..Surgeon…….

19. Pathogenic section: No……

……

20. Complication……

21. Post-operative course……

22. Date of blood collection Day 0……

Day 1……

Day 7……

23. Remarks: ……

Medication Form

Name……..……Age……..years Sex……HN…….....AN……...Ward…….…Bed…..

Medication, Dose, Frequency	Time	Date	Date	Date	Date	Date	Date	Date

Drug allergies…….

……...……

Serum Zinc, Copper, and Chromium Concentrations Recording Form

Pt No.	Serum zinc (μg/dl)			Serum copper (μg/dl)			Serum chromium (μg/l)		
	Day 0	Day 1	Day 7	Day 0	Day 1	Day 7	Day 0	Day 1	Day 7
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									

APPENDIX F

Determination of Serum Zinc, Copper, and Chromium Concentrations

Determination of Serum Zinc Concentration (Hitachi, 1988; Milne, 1994; Milne and Johnson, 1993; Smith et al., 1979)

Reagents

1. Preparation of 0.5 % nitric acid : Concentrated nitric acid 500 µl was diluted to 100 ml with ultrapure water.

2. Preparation of standard zinc solution :

   2.1 Stock standard zinc solution : 1000 mg/l of zinc solution from Merck Co., Germany.

   2.2 Intermediate standard zinc solution :

   Intermediate standard zinc solution I (10 mg/l) : Stock standard zinc solution 1 ml was pipetted into a 100-ml volumetric flask then diluted to volume with ultrapure water. The solution was mixed by inverting at least 10 times.

   Intermediate standard zinc solution II (1 mg/l) : Intermediate standard zinc solution I (10 mg/l) 10 ml was pipetted into a 100-ml volumetric flask then diluted to volume with ultrapure water. The solution was mixed by inverting at least 10 times.

   2.3 Working standard zinc solutions : Various amount of intermediate standard zinc solution II (1 mg/l) were diluted with 0.5 % nitric acid (Table 12) to prepare working standard zinc solution of 0, 250, 500, 750 and 1000 µg/l.

Table 12. The amount of zinc solution and 0.5 % nitric acid used in preparation of working standard zinc solutions.

Working standard zinc solution (µg/l)	0.5 % Nitric acid (µl)	Intermediate standard zinc solution II (µl)	Total volume(µl)
0	1000	0	1000
250	750	250	1000
500	500	500	1000
750	250	750	1000
1000	0	1000	1000

Instrumental Conditions

Serum zinc concentration was determined by graphite furnance atomic absorption spectrophotometer (Model Z-8200 Polarized Zeeman, Hitachi®, Japan) with the following conditions:

Wavelength	307.6 nm
Slit width	1.30 nm
Lamp Current	7.5 mA
Measurement mode	Working curve
Signal mode	Background correction
Carrier gas	200 ml/min

Procedure

1. Instrumental, gas flow settings and aspiration rate were established to optimize signal and minimize background noise. Specific instrumental settings were checked from instrument manual.

2. Standard curve was done from freshly prepared standard solution, presenting the relationship between absorbance and concentration of zinc from measuring standard solutions (Figure 4).

3. Serum samples were taken from deep freezer. Thawed at room temperature and each sample was thoroughly mixed by vortex mixer to obtain homogeneous one.

4. Eight hundred microliters of 0.5 % nitric acid was pipetted into a 1.5-ml plastic microcentrifuge tube. Two hundred microliters of serum sample was added and the solution was immediately mixed thoroughly for 30 seconds. A control serum of Seronorm® was similarly prepared.

5. The standard curve was verified by using Seronorm® before measuring serum samples and after each group of 10 serum samples. The zinc concentration of Seronorm® should be similar and remain constant throughout the analysis within the recommended values.

6. Serum zinc concentration of each sample was analyzed.

7. Serum zinc concentration of each sample was automatically calculated from absorbance readings by interpolation from the standard curve using linear regression equation. Then the concentration was multiplied by 5 to account for sample dilution and expressed in μg/dl.

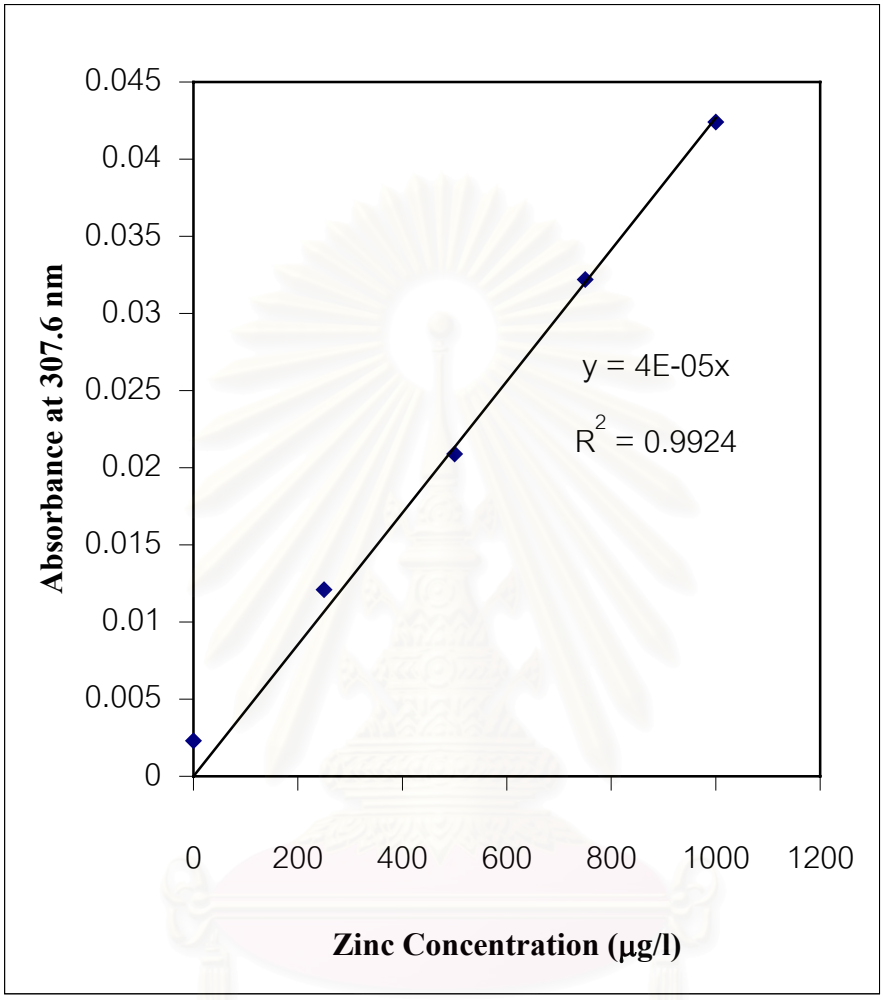

Figure 4. Standard curve of zinc concentration VS absorbance for zinc determination

Determination of Serum Copper Concentration (Hitachi, 1988)

Reagents

1. Preparation of 0.02 N nitric acid : Concentrated nitric acid 136 µl was diluted to 100 ml with ultrapure water.

2. Preparation of standard copper solution :

   2.1 Stock standard copper solution : 1000 mg/l of copper solution from Merck Co., Germany.

   2.2 Intermediate standard copper solution :

   Intermediate standard copper solution I (10 mg/l) : Stock standard copper solution 1 ml was pipetted into a 100-ml volumetric flask then diluted to volume with ultrapure water. The solution was mixed by inverting at least 10 times.

   Intermediate standard copper solution II (1 mg/l) : Intermediate standard copper solution I (10 mg/l) 10 ml was pipetted into a 100-ml volumetric flask then diluted to volume with ultrapure water. The solution was mixed by inverting at least 10 times.

   2.3 Working standard copper solutions : Various amount of intermediate standard copper solution II (1 mg/l) were diluted with 0.02 N nitric acid (Table 13) to prepare working standard copper solution of 0, 50, 100, 200 and 300 µg/l.

Table 13. The amount of copper solution and 0.02 N nitric acid used in preparation of working standard copper solutions.

Working standard copper solution (μg/l)	0.02 N Nitric acid (μl)	Intermediate standard copper solution II (μl)	Total volume (μl)
0	1000	0	1000
50	950	50	1000
100	900	100	1000
200	800	200	1000
300	700	300	1000

Instrumental Conditions

Serum copper concentration was determined by graphite furnance atomic absorption spectrophotometer (Model Z-8200 Polarized Zeeman, Hitachi®, Japan) with the following conditions:

Wavelength	324.8 nm
Slit width	1.30 nm
Lamp Current	7.5 mA
Measurement mode	Working curve
Signal mode	Background correction
Carrier gas	200 ml/min

Procedure

1. Instrumental, gas flow settings and aspiration rate were established to optimize signal and minimize background noise. Specific instrumental settings were checked from instrument manual.

2. Standard curve was done from freshly prepared standard solution, presenting the relationship between absorbance and concentration of copper from measuring standard solutions (Figure 5).

3. Serum samples were taken from deep freezer. Thawed at room temperature and each sample was thoroughly mixed by vortex mixer to obtain homogeneous one.

4. Four hundred microliters of 0.02 N nitric acid was pipetted into a 1.5-ml plastic microcentrifuge tube. One hundred microliters of serum sample was added and the solution was immediately mixed thoroughly for 30 seconds. A control serum of Seronorm® was similarly prepared.

5. The standard curve was verified by using Seronorm® before measuring serum samples and after each group of 10 serum samples. The copper concentration of Seronorm® should be similar and remain constant throughout the analysis within the recommended values.

6. Serum copper concentration of each sample was analyzed.

7. Serum copper concentration of each sample was automatically calculated from absorbance readings by interpolation from the standard curve using linear regression equation. Then the concentration was multiplied by 5 to account for sample dilution and expressed in μg/dl.

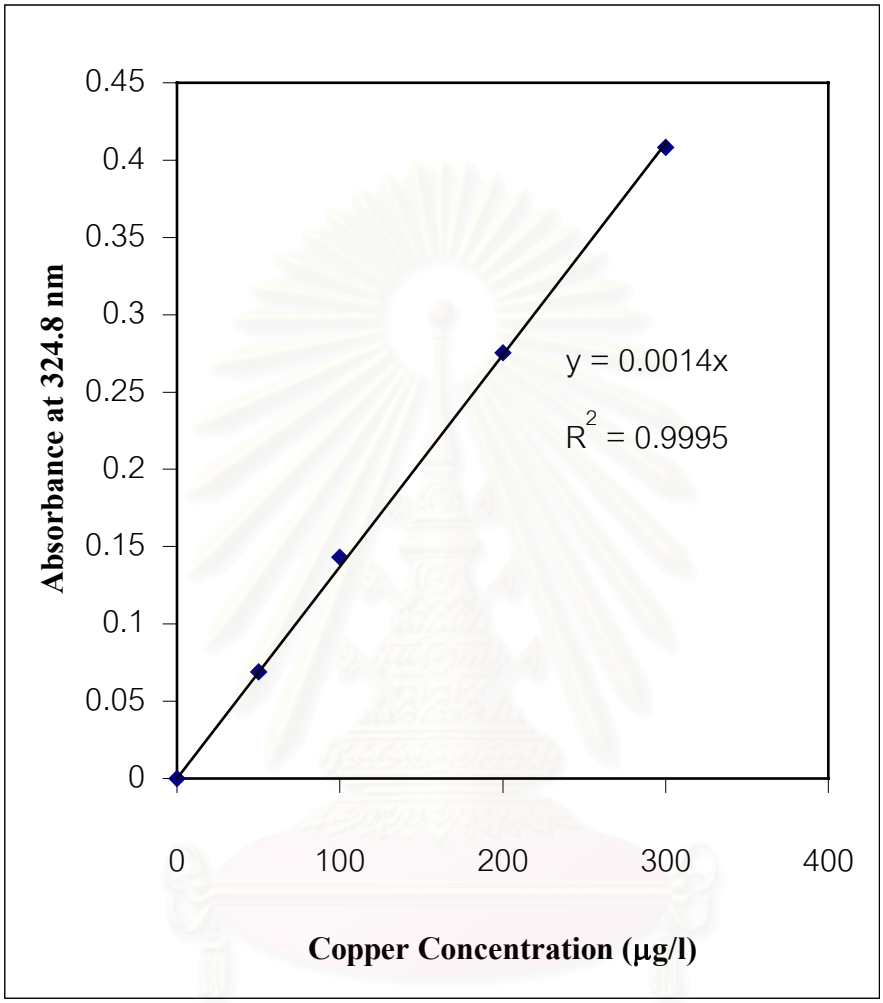

Figure 5. Standard curve of copper concentration VS absorbance for copper determination

Determination of Serum Chromium Concentration (Hitachi, 1988; Nomoto, 1988)

Reagents

1. Preparation of 0.02 N nitric acid : Concentrated nitric acid 136 µl was diluted to 100 ml with ultrapure water.

2. Preparation of standard chromium solution :

   2.1 Stock standard chromium solution : 1000 mg/l of chromium solution from Merck Co., Germany.

   2.2 Intermediate standard chromium solution :

   Intermediate standard chromium solution I (10 mg/l) : Stock standard chromium solution 1 ml was pipetted into a 100-ml volumetric flask then diluted to volume with ultrapure water. The solution was mixed by inverting at least 10 times.

   Intermediate standard chromium solution II (1 mg/l) : Intermediate standard chromium solution I (10 mg/l) 10 ml was pipetted into a 100-ml volumetric flask then diluted to volume with ultrapure water. The solution was mixed by inverting at least 10 times.

   2.3 Working standard chromium solutions : Various amount of intermediate standard chromium solution II (1 mg/l) were diluted with 0.02 N nitric acid (Table 14) to prepare working standard chromium solution of 0, 2, 4, and 6 µg/l.

Table 14. The amount of chromium solution, 0.02 N nitric acid, and Seronorm® used in preparation of working standard chromium solutions.

Working standard chromium solution (µg/l)	0.02 N Nitric acid (µl)	Seronorm® (µl)	Intermediate standard chromium solution II (µl)	Total volume(µl)
0	900	100	0	1000
2	898	100	2	1000
4	896	100	4	1000
6	894	100	6	1000

Instrumental Conditions

Serum chromium concentration was determined by graphite furnance atomic absorption spectrophotometer (Model Z-8200 Polarized Zeeman, Hitachi®, Japan) with the following conditions:

Wavelength	359.3 nm
Slit width	1.30 nm
Lamp Current	10.0 mA
Measurement mode	Simple standard addition
Signal mode	Background correction
Carrier gas	200 ml/min

Procedure

1. Instrumental, gas flow settings and aspiration rate were established to optimize signal and minimize background noise. Specific instrumental settings were checked from instrument manual.

2. Standard curve was done from freshly prepared standard solution, presenting the relationship between absorbance and concentration of chromium from measuring standard solutions (Figure 6).

3. Serum samples were taken from deep freezer. Thawed at room temperature and each sample was thoroughly mixed by vortex mixer to obtain homogeneous one.

4. A control serum was prepared by diluting 200 µl of Seronorm® with 800 µl of 0.02 N nitric acid and the solution was immediately mixed thoroughly for 30 seconds.

5. The standard curve was verified by using Seronorm® before measuring serum samples and after each group of 10 serum samples. The chromium concentration of Seronorm® should be similar and remain constant throughout the analysis within the recommended values.

6. At least 500 µl of serum sample was pipetted into a cup of auto sampler of atomic absorption spectrophotometer and serum chromium concentration of each sample was analyzed.

7. Serum chromium concentration of each sample was automatically calculated from absorbance readings by interpolation from the standard curve using linear regression equation then expressed in µg/l.

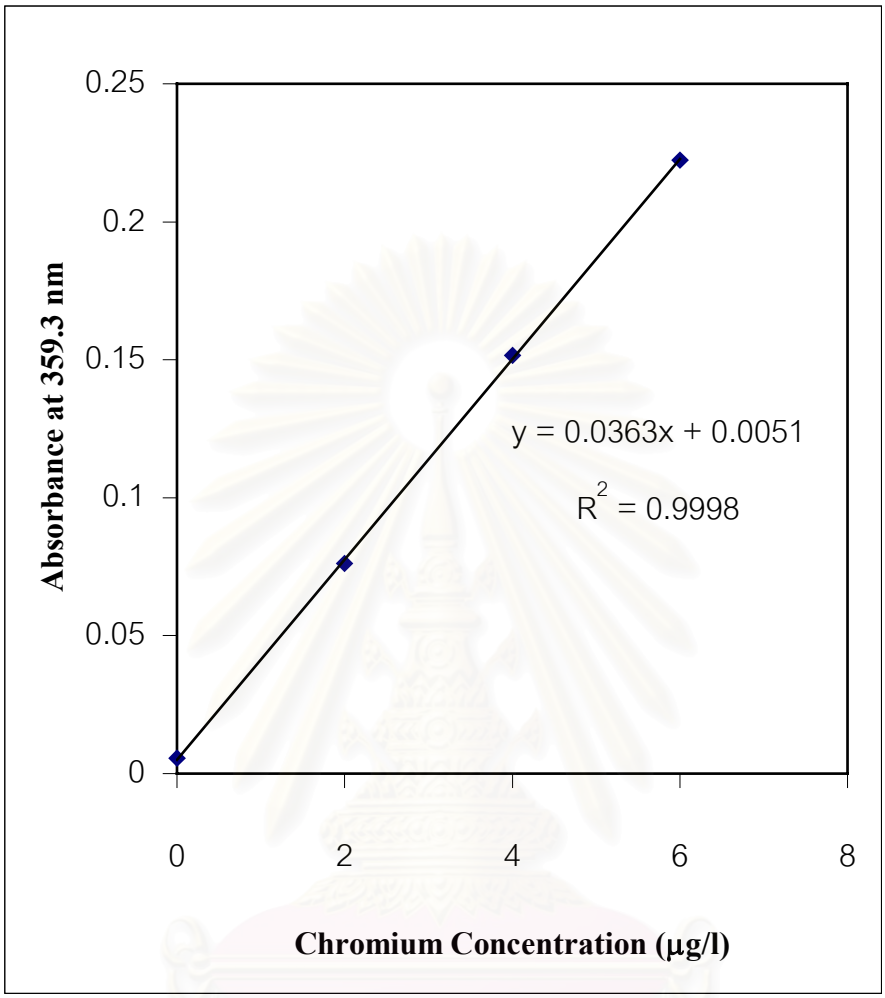

Figure 6. Standard curve of chromium concentration VS absorbance for chromium determination

APPENDIX G

Clinical Characteristics, Serum Zinc, Copper, and Chromium Concentration of Normal Subjects and Cancer Patients

Table 15. Age, weight, height, body mass index, serum zinc, copper, and chromium concentrations of normal subjects

No.	Sex	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m^2)	Serum zinc* (µg/dl)	Serum copper* (µg/dl)	Serum chromium* (µg/l)
1	M	40	55	165	20.20	65.0	85.0	0.60
2	M	59	74	171	25.31	67.5	115.0	0.61
3	M	44	81	173	27.06	82.5	102.5	0.80
4	M	47	70	170	24.22	62.5	115.0	0.63
5	M	45	69	165	25.34	70.0	90.0	0.40
6	M	41	64	162	24.39	77.5	70.0	0.53
7	M	26	52	160	20.31	90.0	140.0	0.71
8	M	47	58	165	21.30	62.5	110.0	0.47
9	M	30	53.5	168	18.96	85.0	97.5	0.61
10	M	30	76	178	23.99	87.5	80.0	0.67
11	M	24	65	170	22.49	85.0	85.0	0.46
12	M	24	66	180	20.37	90.0	75.0	0.53
13	M	43	75	167	26.89	80.0	100.0	0.51
14	M	39	87	180	26.85	112.5	92.5	0.70
15	M	27	66	165	24.24	70.0	120.0	0.50
16	M	38	95	168	33.66	67.5	80.0	0.57
17	M	33	100	180	30.86	100.0	107.5	0.44
18	M	46	62	168	21.97	85.0	80.0	0.38
19	M	37	65	173	21.72	70.0	80.0	0.42
20	M	20	91.5	180	28.24	92.5	60.0	0.44
21	M	19	89.5	174	29.56	105.0	75.0	0.36
22	M	33	68	179	21.22	75.0	70.0	0.34
23	M	28	55	164	20.45	70.0	77.5	0.33

* Each value represents a mean of duplicate tube

M = Male, F = Female

Table 15. Age, weight, height, body mass index, serum zinc, copper, and chromium concentrations of normal subjects (continued)

No.	Sex	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m^2)	Serum zinc* (µg/dl)	Serum copper* (µg/dl)	Serum chromium* (µg/l)
24	M	32	68	165	24.98	87.5	72.5	0.35
25	M	25	55	170	19.03	95.0	82.5	0.41
26	M	47	87	167	31.20	55.0	105.0	0.32
27	M	23	75	165	27.55	82.5	60.0	0.48
28	M	33	73	180	22.53	80.0	77.5	0.37
29	M	30	65	173	21.72	107.5	75.0	0.47
30	M	40	66.5	165	24.43	130.0	95.0	0.76
31	F	40	65	158	26.04	70.0	90.0	0.40
32	F	45	55	155	22.89	75.0	97.5	0.48
33	F	41	61	158	24.44	77.5	75.0	0.41
34	F	22	65	165	23.88	85.0	82.5	0.28
35	F	28	65	156	26.71	77.5	75.0	0.29
36	F	18	47	159	18.59	85.0	92.5	0.39
37	F	26	51	158	20.43	77.5	85.0	0.41
38	F	42	49	151	21.49	65.0	70.0	0.32
39	F	31	46	148	21.00	80.0	120.0	0.26
40	F	42	52	152	22.51	72.5	97.5	0.52
41	F	39	67	157	27.18	60.0	95.0	0.41
42	F	47	56	161	21.60	100.0	95.0	0.28
43	F	34	60	160	23.44	82.5	102.5	0.23
44	F	36	64	160	24.25	90.0	110.0	0.37
45	F	24	50	155	20.81	65.0	77.5	0.19
46	F	35	59	163	22.21	72.5	90.0	0.29

***** Each value represents a mean of duplicate tube

M = Male, F = Female

Table 15. Age, weight, height, body mass index, serum zinc, copper, and chromium concentrations of normal subjects (continued)

No.	Sex	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m^2)	Serum zinc* (µg/dl)	Serum copper* (µg/dl)	Serum chromium* (µg/l)
47	F	39	55	160	21.48	87.5	95.0	0.23
48	F	24	63	173	21.05	95.0	75.0	0.54
49	F	20	53	165	19.47	90.0	60.0	0.52
50	F	19	56.5	164	21.01	110.0	70.0	0.47
51	F	26	47	155	19.56	120.0	75.0	0.70
52	F	36	69.5	162	26.48	90.0	100.0	0.56
53	F	21	47	159	18.59	105.0	75.0	0.50
54	F	22	49	157	19.88	75.0	80.0	0.61
55	F	18	54	164	20.08	125.0	77.5	0.42
56	F	38	68	158	27.24	92.5	110.0	0.35
57	F	42	47.5	158	19.03	87.5	82.5	0.79
58	F	52	61	165	22.41	110.0	90.0	0.44
59	F	34	49	156	20.13	82.5	105.0	0.48
60	F	18	49	155	20.40	80.0	120.0	0.93
Range		18-59	46-100	148-180	18.59-33.66	55-130	60-140	0.19-0.93
Mean ± SD		33.48 ± 9.87	63.46 ± 12.82	164.62 ± 7.99	23.26 ± 3.42	84.58 ± 16.21	89.08 ± 16.94	0.47 ± 0.15

***** Each value represents a mean of duplicate tube

M = Male, F = Female

Table 16. Clinical characteristics and surgical treatment of individual cancer patients

Patient No.	Sex	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m^2)	Diagnosis	**Operation**
1	M	43	58	170	20.07	Cancer of rectum	Abdomino-peritoneal resection
2	F	77	42	150	18.67	Cancer of ascending colon with partial gut obstruction	Right half colectomy
3	M	39	68	160.5	26.40	Recurrent cancer of nasopharynx S/P radiation, and chemotherapy	Gastrostomy
4	M	71	51	159	20.17	Cancer of cervical esophagus	Gastrostomy
5	M	37	55	160	21.48	Hepatocellular carcinoma, left lobe	Left extended hepatectomy
6	M	72	45	165	16.53	Cancer head of pancreas, periampullary cancer	Cholecystojejunostomy with jejunojejunostomy with gastrojejunostomy with liver biopsy
7	F	80	52	155	21.64	Cancer of upper rectum	Low anterior resection with end to end colorectal anastomosis
8	F	51	43	156	17.67	Recurrent cancer of stomach S/P subtotal gastrectomy	Total gastrectomy with spleenectomy with end to side esophagojejunostomy and end to side jejunojejunostomy

M = Male, F= Female

BMI = Body mass index

Table 16. Clinical characteristics and surgical treatment of individual cancer patients (continued)

Patient No.	Sex	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m^2)	Diagnosis	Operation
9	F	62	42	157	17.04	Rectosigmoid carcinoma	Low anterior resection
10	M	55	56	160	21.88	Cancer ampulla of vater with obstructive jaundice	Choledochojejunostomy, jejunostomy
11	M	43	46	165	16.90	Cancer of esophagus	Gastrostomy
12	M	67	47	183	14.03	Cancer of floor of mouth with cancer of esophagus	Gastrostomy
13	F	65	48.5	144	23.39	Adenocarcinoma of lower rectum	Abdomino-peritoneal resection
14	M	60	50	169	17.51	Cancer of rectum (partial gut obstruction)	Abdomino-peritoneal resection
15	M	56	49	163.5	18.33	Cancer of cervical esophagus	Gastrostomy
16	F	69	46	149.5	20.58	Cancer of rectum	Abdomino-peritoneal resection with colostomy
17	F	39	60	149	27.03	Cancer of rectum	Abdomino-peritoneal resection with end colostomy
18	M	61	51.5	164	19.15	Cancer of transverse colon	Exploratory laparotomy with extended right hemicolectomy

M = Male, F= Female

BMI = Body mass index

Table 16. Clinical characteristics and surgical treatment of individual cancer patients (continued)

Patient No.	Sex	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m^2)	Diagnosis	Operation
19	M	53	72	167	25.82	Cancer of rectosigmoid colon	Anterior resection, partial cystectomy
20	F	54	45	147.5	20.68	Cancer of rectum with liver metastasis	Abdomino-peritoneal resection and left lateral liver segmentectomy
21	M	53	46	152.5	19.78	Cholangiocarcinoma	Tumor resection with Roux-en-Y hepaticojejunostomy, sacrificed right hepatic artery
22	M	59	58	169.5	20.19	Cancer of esophagus with dysphagia	Gastrostomy
23	F	62	55	156	22.60	Hepatocellular carcinoma with metastasis	Exploratory laparotomy with gastroduodenoscope
24	M	50	49	167	17.57	Cancer of sigmoid colon with bony metastasis	Transverse loop colostomy
25	M	54	65	167	23.31	Cancer head of pancreas, paraganglioma	Exploratory laparotomy with biopsy suprapancreatic node and cholecystostomy
26	M	48	53	161.5	20.32	Cancer of ampulla locally advanced	Double by pass (Roux-en-Y cholecystojejunostomy, gastrojejunostomy)
27	M	55	59	163	22.21	Cancer of esophagus	Esophagectomy with gastric interposition and feeding jejunostomy

M = Male, F= Female

BMI = Body mass index

Table 16. Clinical characteristics and surgical treatment of individual cancer patients (continued)

Patient No.	Sex	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m^2)	Diagnosis	Operation
28	M	54	40	164	14.87	Cancer of cardia	Exploratory laparotomy with gastrostomy
29	F	49	39.5	152.5	16.98	Cancer of esophagus	Gastrostomy
30	F	62	45	152	19.48	Cancer of sigmoid colon	Tumor resection with Hartmann's procedure (sigmoidectomy with colostomy)
31	M	71	60	165.5	21.91	Cancer of lower rectum	Abdomino-peritoneal resection
32	M	47	47.5	162.5	17.99	Tumor head of pancreas	Pyrolic preserving pancreaticoduodenectomy
33	F	61	40	150	17.78	Cancer of esophagus	Gastrostomy
34	M	61	70	163	26.35	Cancer of rectum	Exploratory laparotomy with abdomino-peritoneal resection
35	F	53	78	152	33.76	Cancer head of pancreas	Exploratory laparotomy with double bypass with cholecystectomy (Roux-en-Y choledochojejunostomy and gastrojejunostomy)
36	M	58	49	162	18.67	Cholangiocarcinoma	Exploratory laparotomy simple suture with omental graft biopsy ulceration rime

M = Male, F= Female

BMI = Body mass index

Table 16. Clinical characteristics and surgical treatment of individual cancer patients (continued)

Patient No.	Sex	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m^2)	Diagnosis	Operation
37	M	48	63	170.5	21.67	Cancer of caecum	Right half colectomy with end to end anastomosis
38	F	74	40	137	21.31	Cancer of rectum with adenomatus polyp at descending colon	Left half colectomy with low anterior resection
39	M	54	55	168	19.49	Left lobe cholangiocarcinoma	Exploratory laparotomy with cholecystectomy and intraoperative cholangiography (IOC) and liver biopsy
40	F	73	34	140	17.35	Recurrent cancer of stomach with incisional hernia S/P subtotal gastrectomy	Jejunojejunostomy (Brown loop) with Iliotransverse colostomy with repair incisional hernia
41	F	53	46	148	21.00	Recurrent cancer of colon at caecum S/P abdomina-peritoneal resection and left hemicolectomy	Exploratory laparotomy with lysis adhesion and right hemicolectomy and end iliostomy
42	F	54	40	145	19.02	Cholangiocarcinoma of right lobe liver with rupture duodenum 2^{nd} part	Exploratory laparotomy with repair duodenum

M = Male, F= Female

BMI = Body mass index

Table 16. Clinical characteristics and surgical treatment of individual cancer patients (continued)

Patient No.	Sex	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m^2)	Diagnosis	Operation
43	M	52	56	150	24.89	Cancer of ascending colon	Right hemicolectomy
44	F	70	58	146.5	27.02	Cholangiocarcinoma with obstruction of common hepatic and left hepatic duct	Exploratory common bile duct with choledochoscopy with retain T-tube and repair duodenum

M = Male, F= Female

BMI = Body mass index

Table 17. Serum zinc, copper, and chromium concentrations of cancer patients before and after operation

Patient No.	Serum zinc* (μg/dl)			Serum copper* (μg/dl)			Serum chromium* (μg/l)		
	Day 0	Day 1	Day 7	Day 0	Day 1	Day 7	Day 0	Day 1	Day 7
1	72.5	40.0	82.5	100.0	70.0	120.0	0.61	0.28	0.36
2	47.5	40.0	32.5	115.0	110.0	95.0	0.53	0.52	0.19
3	127.5	110.0	150.0	192.5	205.0	160.0	0.61	0.06	0.08
4	85.0	60.0	92.5	132.5	132.5	140.0	0.18	0.13	0.16
5	52.5	195.0	240.0	162.5	60.0	112.5	0.50	0.11	0.50
6	117.5	192.5	235.0	182.5	170.0	162.5	0.33	0.17	0.40
7	135.0	112.5	152.5	135.0	72.5	90.0	0.32	0.17	0.39
8	137.5	102.5	135.0	52.5	60.0	72.5	0.13	0.10	0.22
9	150.0	112.5	175.0	157.5	130.0	152.5	0.25	0.14	0.30
10	150.0	140.0	152.5	250.0	170.0	220.0	0.25	0.11	0.24
11	102.5	100.0	135.0	160.0	160.0	167.5	0.24	0.18	0.15
12	145.0	120.0	140.0	110.0	105.0	92.5	0.43	0.15	0.30
13	155.0	135.0	150.0	112.5	82.5	115.0	0.34	0.07	0.10
14	157.5	142.5	115.0	170.0	122.5	130.0	0.24	0.10	0.14
15	105.0	95.0	167.5	160.0	160.0	185.0	0.06	0.06	0.05
16	140.0	10.0	55.0	105.0	75.0	125.0	0.12	0.14	0.14
17	150.0	45.0	170.0	110.0	82.5	137.5	0.13	0.11	0.21
18	155.0	150.0	187.5	132.5	140.0	160.0	0.16	0.13	0.29
19	105.0	45.0	85.0	145.0	157.5	135.0	0.16	0.13	0.08
20	92.5	57.5	70.0	135.0	85.0	130.0	0.32	0.11	0.29

* Each value represents a mean of duplicate tube

Collected blood samples on the day before operation

Collected blood samples on the first day after operation

Collected blood samples on the seventh day after operation

Table 17. Serum zinc, copper, and chromium concentrations of cancer patients before and after operation (continued)

Patient No.	Serum zinc* (μg/dl)			Serum copper* (μg/dl)			Serum chromium* (μg/l)		
	Day 0	Day 1	Day 7	Day 0	Day 1	Day 7	Day 0	Day 1	Day 7
21	87.5	105.0	47.5	252.5	165.0	180.0	0.18	0.06	0.11
22	107.5	90.0	47.5	160.0	150.0	182.5	0.10	0.07	0.09
23	120.0	30.0	75.0	147.5	95.0	125.0	0.09	0.02	0.28
24	147.5	45.0	42.5	205.0	185.0	177.5	0.50	0.11	0.17
25	90.0	80.0	50.0	260.0	225.0	255.0	0.21	0.20	0.09
26	65.0	60.0	10.0	305.0	232.5	230.0	0.21	.013	0.20
27	130.0	15.0	45.0	160.0	85.0	175.0	0.21	0.13	0.13
28	100.0	35.0	32.5	145.0	130.0	120.0	0.12	0.10	0.15
29	52.5	17.5	50.0	167.5	140.0	155.0	0.22	0.12	0.22
30	85.0	20.0	7.5	150.0	150.0	160.0	0.27	.018	0.15
31	15.0	5.0	20.0	80.0	72.5	90.0	0.37	0.31	0.23
32	30.0	12.5	35.0	167.5	150.0	155	0.15	0.08	0.34
33	42.5	17.5	35.0	105.0	110.0	112.5	0.24	0.19	0.14
34	57.5	5.0	27.5	75.0	65.0	70.0	0.33	0.20	0.10
35	37.5	15.0	40.0	190.0	135.0	160.0	0.43	0.22	0.41
36	20.0	22.5	40.0	245.0	217.5	232.5	0.17	0.10	0.15
37	25.0	35.0	67.5	147.5	135.0	140.0	0.18	0.15	0.21
38	32.5	12.5	35.0	105.0	95.0	100.0	0.18	0.13	0.22
39	32.0	21.5	35.0	160.0	130.0	147.5	0.35	0.11	0.12
40	26.0	25.5	46.0	100.0	100.0	102.5	0.22	0.18	0.23
41	38.5	26.0	50.0	115.0	125.0	140.0	0.23	0.17	0.16

* Each value represents a mean of duplicate tube

Collected blood samples on the day before operation

Collected blood samples on the first day after operation

Collected blood samples on the seventh day after operation

Table 17. Serum zinc, copper, and chromium concentrations of cancer patients before and after operation (continued)

Patient No.	Serum zinc* (μg/dl)			Serum copper* (μg/dl)			Serum chromium* (μg/l)		
	Day 0	Day 1	Day 7	Day 0	Day 1	Day 7	Day 0	Day 1	Day 7
42	33.5	8.0	31.5	152.5	110.0	95.0	0.17	0.11	0.19
43	28.0	30.5	34.5	130.0	147.5	145.0	0.27	0.13	0.18
44	34.0	20.0	12.0	185.0	147.5	200.0	0.22	0.11	0.21
Range	15-157.5	5-195	7.5-240	52.5-305	60-235	70-255	0.06-0.61	0.02-0.52	0.05-0.50
Mean ± SD	86.58 ± 49.97	62.70 ± 52.46	82.70 ± 62.49	152.95 ± 51.81	128.35 ± 44.68	144.38 ± 42.60	0.26 ± 0.14	0.14 ± 0.08	0.21 ± 0.10

* Each value represents a mean of duplicate tube

Collected blood samples on the day before operation

Collected blood samples on the first day after operation

Collected blood samples on the seventh day after operation

Table 18. Number of cancer patients categorized by aggressiveness and prognosis of the disease

Categories of the cancer disease	Males	Females	Total
Group 1	9	10	**19**
Cancer of rectum	4	5	9
Cancer of rectum with liver metastasis	-	1	1
Cancer of ascending colon with partial gut obstruction	1	1	2
Cancer of rectosigmoid colon	2	2	4
Cancer of transverse colon	1	-	1
Cancer of caecum	1	-	1
Recurrent cancer of colon and caecum	-	1	1
Group 2	8	4	**12**
Recurrent cancer of nasopharynx	1	-	1
Cancer of esophagus	5	2	7
Cancer of floor of mouth with cancer of esophagus	1	-	1
Recurrent cancer of stomach	-	2	2
Cancer of cardia	1	-	1
Group 3	9	4	**13**
Hepatocellular carcinoma	1	1	2
Cholangiocarcinoma	3	2	5
Cancer head of pancreas, periampullary cancer	2	1	3
Cancer ampulla of vater with obstructive jaundice	2	-	2
Tumor of head of pancreas	1	-	1

Table 19. Number of cancer patients categorized by the extent of surgery

Categories of surgery	Males	Females	Total
Group 1	8	3	**11**
Gastrostomy	6	2	8
Exploratory laparotomy with gastrostomy	1	-	1
Exploratory laparotomy with gastroduodenoscope	-	1	1
Transverse loop colostomy	1	-	1
Group 2	11	10	**21**
Right, left half colectomy	1	1	2
Low anterior resection	1	2	3
Left half colectomy with low anterior resection	-	1	1
Left extended hepatectomy	1	-	1
Cholecystojejunostomy, jejunojejunostomy, gastrojejunostomy	2	1	3
Right hemicolectomy	2	1	3
Exploratory laparotomy with biopsy suprapancreatic node	1	-	1
Double bypass	1	1	2
Hartmann's procedure	-	1	1
Exploratory laparotomy simple suture with omental graft biopsy	1	-	1
Exploratory laparotomy with cholecystectomy	1	-	1
Exploratory laparotomy with repair duodenum	-	1	1
Exploratory common bile duct with choledochoscopy	-	1	1

Table 19. Number of cancer patients categorized by the extent of surgery (continued)

Categories of surgery	Males	Females	Total
Group 3	7	5	**12**
Abdomino-peritoneal resection	4	4	8
Total gastrectomy, spleenectomy, esophagojejunostomy, jejunojejunostomy	-	1	1
Tumor resection with hepaticojejunostomy, sacrificed right hepatic artery	1	-	1
Esophagectomy with gastric interposition, jejunostomy	1	-	1
Pyrolic preserving pancreaticoduodenectomy	1	-	1

APPENDIX H

Graphical Display of the Results

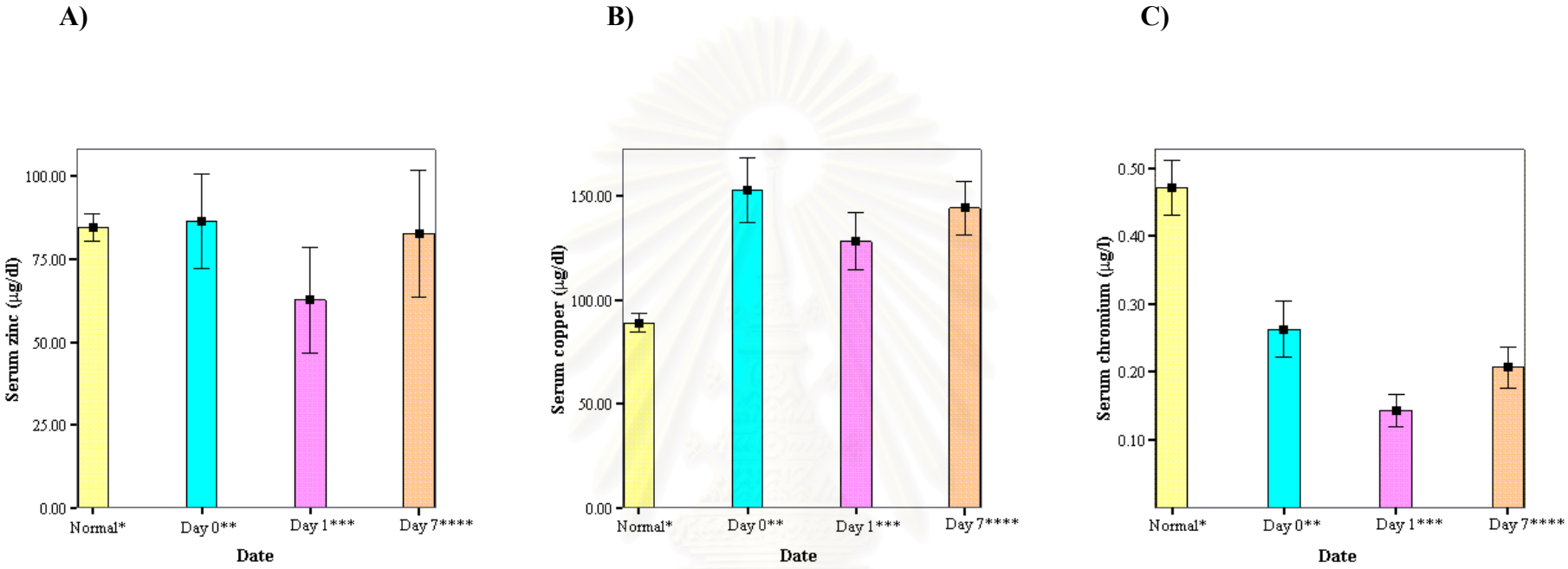

Figure 7. Means serum concentration of A) zinc, B) copper, and C) chromium of cancer patients before and after operation compared with those in normal subjects (black dots show mean, error bars show 95% confidence interval of mean, * normal subjects, ** collected blood samples on the day before operation, *** collected blood samples on the first day after operation, **** collected blood samples on the seventh day after operation)

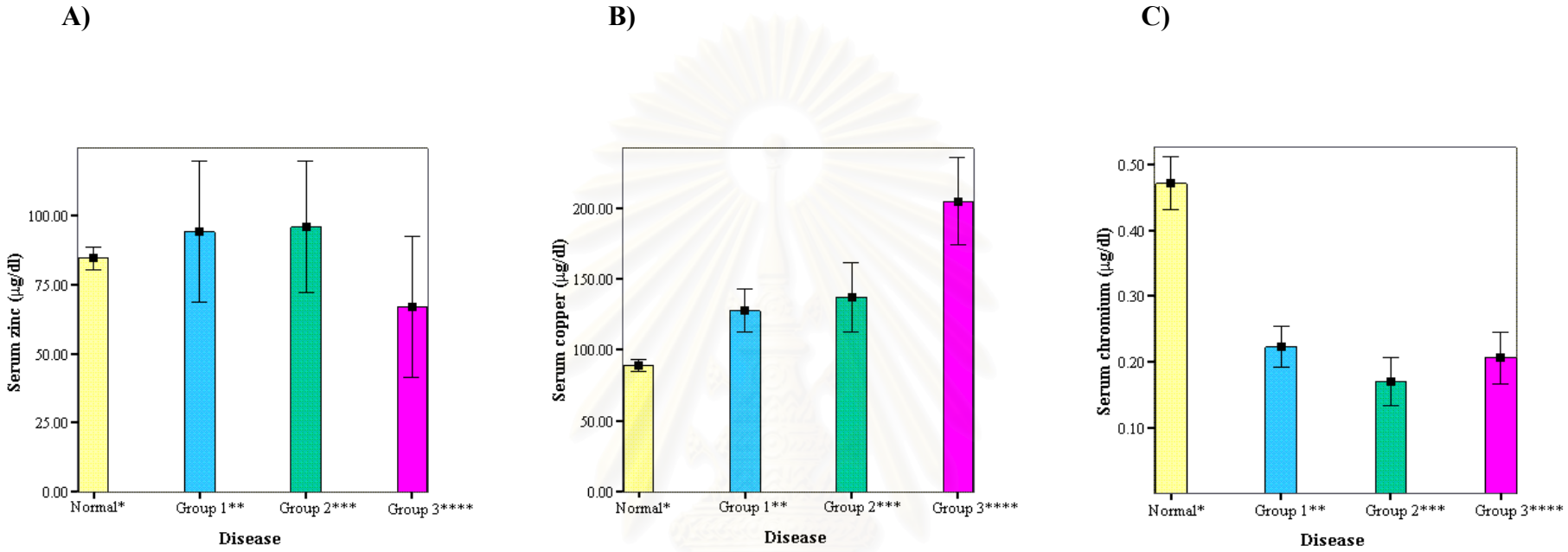

Figure 8. Means serum concentration of A) zinc, B) copper, and C) chromium of normal subjects and cancer patients categorized by aggressiveness and prognosis of the disease obtained from Day 0 (black dots show mean, error bars show 95% confidence interval of mean, * normal subjects, ** patients who have no associated disphagia, relatively less aggressive tumor with reasonable survival, *** patients who have associated disphagia, relatively aggressive tumor with poor survival, **** patients who have no associated disphagia, relatively aggressive tumor with poor survival)

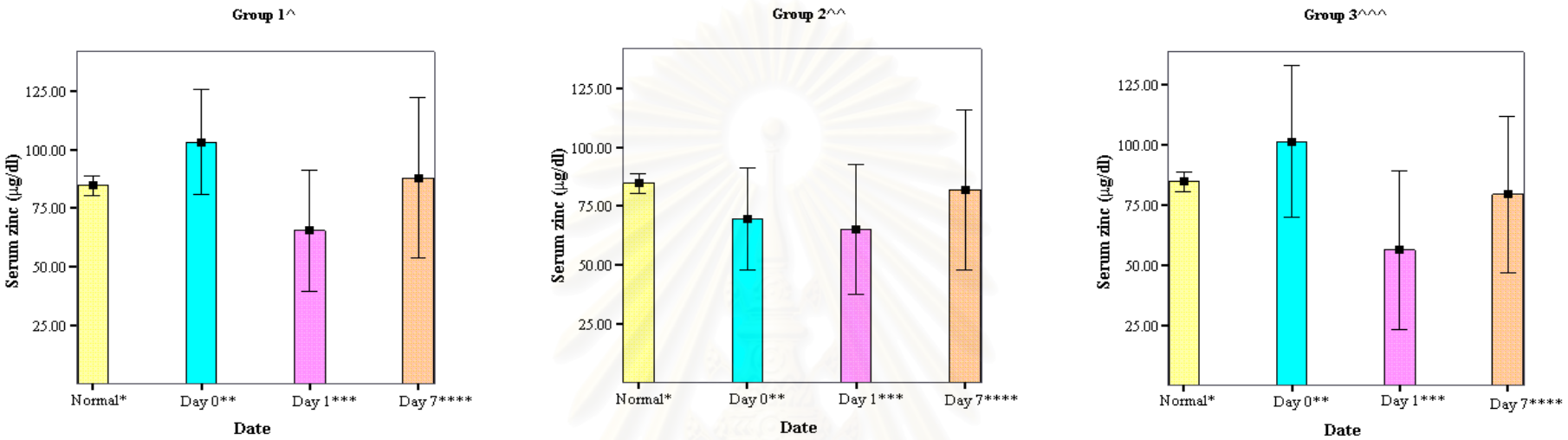

Figure 9A. Means serum zinc concentration of normal subjects and cancer patients categorized by the extent of surgery (black dots show mean, error bars show 95% confidence interval of mean, * normal subjects, ** collected blood samples on the day before operation, *** collected blood samples on the first day after operation, **** collected blood samples on the seventh day after operation, ^ patients who received relatively minor operation-operative time < 1 hr, ^^ patients who received major operation-operative time 1 - 2 hr, ^^^ patients who received major operation-operative time > 2 hr, have extensive organ dissection and resection)

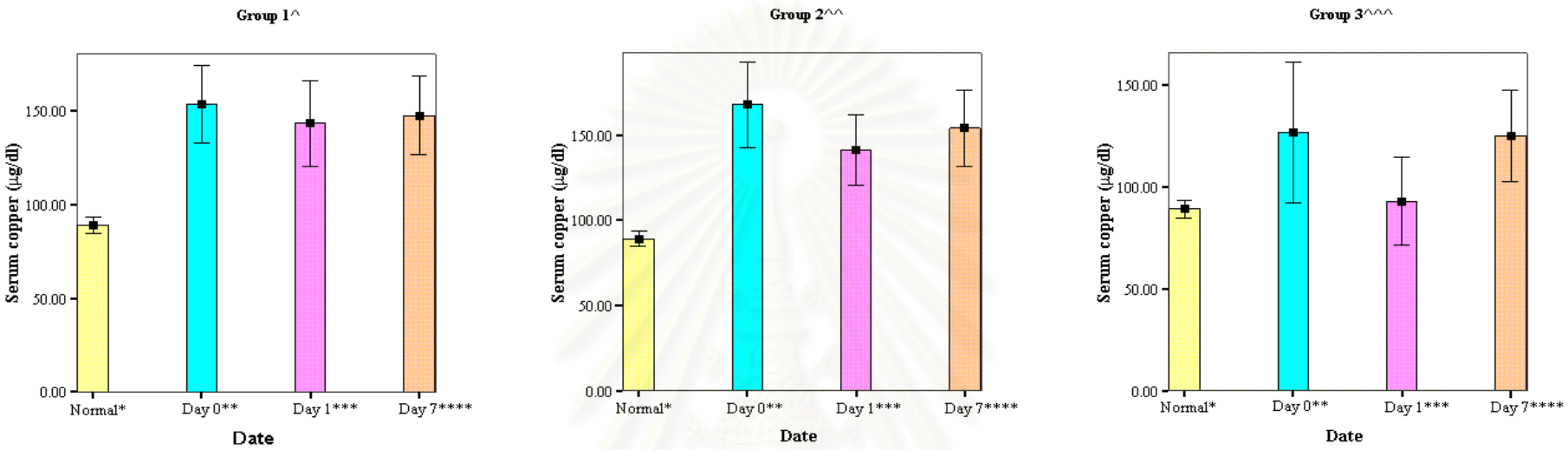

Figure 9B. Means serum copper concentration of normal subjects and cancer patients categorized by the extent of surgery (black dots show mean, error bars show 95% confidence interval of mean, * normal subjects, ** collected blood samples on the day before operation, *** collected blood samples on the first day after operation, **** collected blood samples on the seventh day after operation, ^ patients who received relatively minor operation-operative time < 1 hr, ^^ patients who received major operation-operative time 1 - 2 hr, ^^^ patients who received major operation-operative time > 2 hr, have extensive organ dissection and resection)

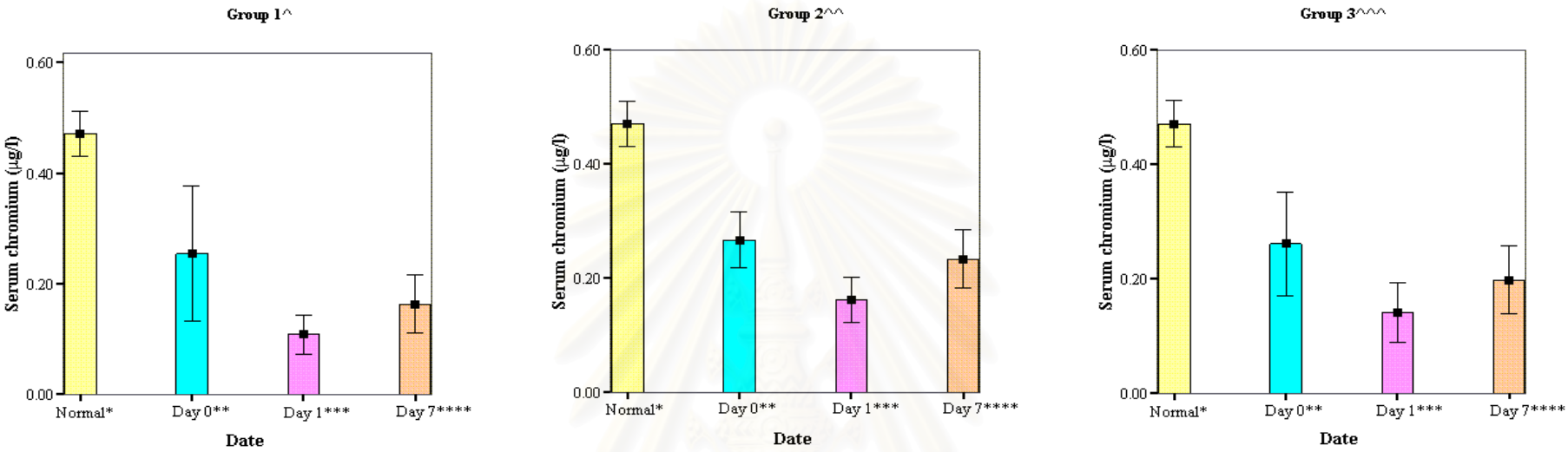

Figure 9C. Means serum chromium concentration of normal subjects and cancer patients categorized by the extent of surgery (black dots show mean, error bars show 95% confidence interval of mean, * normal subjects, ** collected blood samples on the day before operation, *** collected blood samples on the first day after operation, **** collected blood samples on the seventh day after operation, ^ patients who received relatively minor operation-operative time < 1 hr, ^^ patients who received major operation-operative time 1 - 2 hr, ^^^ patients who received major operation-operative time > 2 hr, have extensive organ dissection and resection)

APPENDIX I

Statistical Analysis

1. Independent Sample t-test

Hypothesis

H_0 : no difference in the mean of the two samples

H_A : mean of sample 1 is higher (lower) than sample 2

Table 20. Test statistic (Independent sample t-test) information for the means serum zinc, copper, and chromium

Variables	Serum zinc			Serum copper			Serum chromium		
	t	df	p-value	t	df	p-value	t	df	p-value
Normal subjects VS entire population of cancer patients (Day 0)	-0.27	102	0.394	-7.875	102	0.000	7.149	102	0.000
Normal subjects VS entire population of cancer patients (Day 1)	2.675	102	0.005	-5.545	102	0.000	14.029	102	0.000
Normal subjects VS entire population of cancer patients (Day 7)	0.195	102	0.423	-8.15	102	0.000	10.515	102	0.000
Normal subjects VS cancer patients in disease group 1	-0.774	77	0.224	-5.128	77	0.000	4.55	77	0.000
Normal subjects VS cancer patients in disease group 2	-1.024	70	0.163	-4.217	70	0.0005	4.928	70	0.000
Normal subjects VS cancer patients in disease group 3	1.491	71	0.08	-8.056	71	0.000	4.814	71	0.000
Cancer patients in disease group 1 VS group 2	-0.108	29	0.4575	-0.747	29	0.2305	1.138	29	0.132
Cancer patients in disease group 1 VS group 3	1.546	30	0.0495	-4.844	30	0.000	0.839	30	0.204
Cancer patients in disease group 2 VS group 3	1.811	23	0.0415	-3.7	23	0.0005	-0.382	23	0.353

2. Paired Sample t-test

Hypothesis

H_0 : no difference in the mean difference of the two samples (before and after)

H_A : mean difference lower in sample 2 (after treatment)

Table 21. Test statistic (Paired samples t-test) information for the means serum zinc, copper, and chromium

Groups	Paired-variables	Serum zinc			Serum copper			Serum chromium		
		t	df	p-value	t	df	p-value	t	df	p-value
Entire population of cancer patients	Day 0 VS Day 1	3.504	43	0.0005	5.574	43	0.000	6.572	43	0.000
	Day 0 VS Day 7	0.505	43	0.308	2.166	43	0.018	2.652	43	0.0055
Cancer patients in surgery group 1	Day 0 VS Day 1	3.774	10	0.002	1.888	10	0.044	2.676	10	0.0115
	Day 0 VS Day 7	1.025	10	0.1645	0.954	10	0.181	1.564	10	0.0745
Cancer patients in surgery group 2	Day 0 VS Day 1	0.48	20	0.318	3.735	20	0.0005	5.288	20	0.000
	Day 0 VS Day 7	-1.015	20	0.161	2.348	20	0.0145	1.435	20	0.0835
Cancer patients in surgery group 3	Day 0 VS Day 1	3.348	11	0.0035	4.245	11	0.0005	3.97	11	0.001
	Day 0 VS Day 7	2.113	11	0.029	0.198	11	0.423	1.56	11	0.0735

BIOGRAPHY

Miss Nualnit Wichien was born on February 14, 1973 in Bangkok, Thailand. She received her Bachelor of Science in Pharmacy Degree from the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand in 1996. After graduation, she works at the Pharmacy Department, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand.