Journal of Food and Drug Analysis
1994. 2(3) : 211-216

藥物食品分析
第二卷　第三期

肝細胞癌患者腫瘤與非腫瘤組織中四種金屬離子濃度之測定

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摘　　要

本篇利用感應耦合電漿原子發射光譜法來測定肝細胞癌組織中四種金屬離子：鋅、錳、鐵、銅之濃度，分析18位原發性肝細胞癌患者經開刀所取出之腫瘤組織，並以同患者之非腫瘤組織為對照，發現腫瘤組織中鋅、錳離子之含量較非腫瘤組織低($P<0.05$及$P<0.0005$)，推測其原因可能與腫瘤組織中以鋅，錳離子為輔因子之某些葡萄糖代謝酵素，如lactate dehydrogenase, glucokinase, pyruvate dehydrogenase之活性降低有關，而鐵和銅離子則在兩區域間無統計意義之差別。

前　　言

生物體內有許多微量金屬元素參與不同的生理代謝活動，且扮演極重要的調節作用。幾乎體內四分之一以上的酵素本身與金屬離子鍵結或是需要它們的參與才能產生活性[1－3]，其中鋅、錳、鐵、銅則被視為體內必要性的金屬離子[4]。鋅離子為許多肝臟酵素之輔因子，如乳酸脫氫酶(lactate dehydrogenase)，果糖1,6二磷酸酶(fructose-1,6-bisphosphatase)，鹼性磷酸酶(alkaline phosphatase)以及醇脫氫酶(alcohol dehydrogenase)。錳離子則為丙酮酸羧化酶(pyruvate carboxylase)及葡萄糖激酶(glucokinase)等酵素之輔因子。鐵離子參與體內氧氣的輸送（血紅蛋白與肌紅蛋白）及電子傳遞（細胞色素）。銅離子亦為許多酵素不可或缺的重要元素，如細胞色素氧化酶(cytochrome oxidase)。既然這四種金屬離子在正常生理代謝功能上擔任如此重要之角色，它們與人類疾病間的關係便非常值得被探討。

肝細胞癌(hepatocellular carcinoma, HCC)是國人十大死亡原因之一，其發生原因及腫瘤生化學是近年來研究的熱門話題[5－10]，但有關金屬離子與HCC代謝間關係的文獻並不多見。Okuno等人曾以中子活化分析(neutron activation analysis)研究肝癌組織中微量金屬的含量，結果發現肝癌組織中鋅、錳離子濃度要比腫瘤周圍的肝組織為低[11]。而更早期的Wright等人利用原子吸收光譜(atomic-absorption spectroscopy)研究肝病變中鋅離子的濃度變化，亦得到類似的結果[12]。此外，由磁振造影(magnetic resonance imaging,MRI)的結果，發現在肝癌腫瘤組織似有銅離子的堆積[13,14]。

感應耦合電漿原子發射光譜儀(lnductively Coupled Plasma-Atomic Emission Spectroscopy,ICP-AES)是一種以氬氣電漿作為激發源，連接精密的分光儀組合而成，具有高靈敏度（測至ppb）及同時測定多種元素的優點。曾有人應用此儀器測定肝癌患者及健康人血清中15種金屬元素的濃度並分析其差異性[15]。本研究則利用ICP-AES分析並比較HCC患者肝癌組織與本身非腫瘤肝組織間可溶於水的鋅、錳、鐵、銅離子濃度之差異，並將其結果與血液生化檢查作一相關性的探討。

材料與方法

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一、檢體來源

18位肝細胞癌患者(其中男性13位，女性5位，年齡層分佈於30-72歲)，於民國80-82年間經台中榮民總醫院診斷爲肝癌且施以外科手術，所切下的組織經由病理切片證實爲HCC，對照組爲同患者之非腫癌肝組織。

二、檢體處理

手術所取得之組織由專業醫師分爲腫瘤組織與非腫瘤組織兩部分，以去離子水洗去檢體表面的血水後以吸水紙吸去多餘的水份，精確秤重。加上檢體重量5倍之去離子水充分絞碎後離心(4 °C, 1700xg,30 min)，上清液冷凍乾燥後於－70 °C下保存，測定前再以去離子水溶解稀釋至適當濃度。故本實驗所測得之結果爲組織中可溶於水的金屬離子濃度。

三、儀器及分析條件

(一)儀器

ICP-AES, Plasmakon S-35, Kontron, West Germany

(二)分析條件

radio frequency output : 1.7 KW
argon coolant gas input rate : 1.5 l/min
argon plasma gas flow rate : 0.3 l/min
carrier gas flow rate : 1.2 l/min
analytical lines : Zn (213.856 nm), Fe (259.940 nm), Mn (260.569 nm), Cu (324.754 nm)

四、數據之處理

利用Student's paired t-test判定腫瘤與非腫瘤組織間金屬離子濃度是否有統計上有意義之差別，而其與血液生化檢查之相關性則以simple regression program計算。

結果與討論

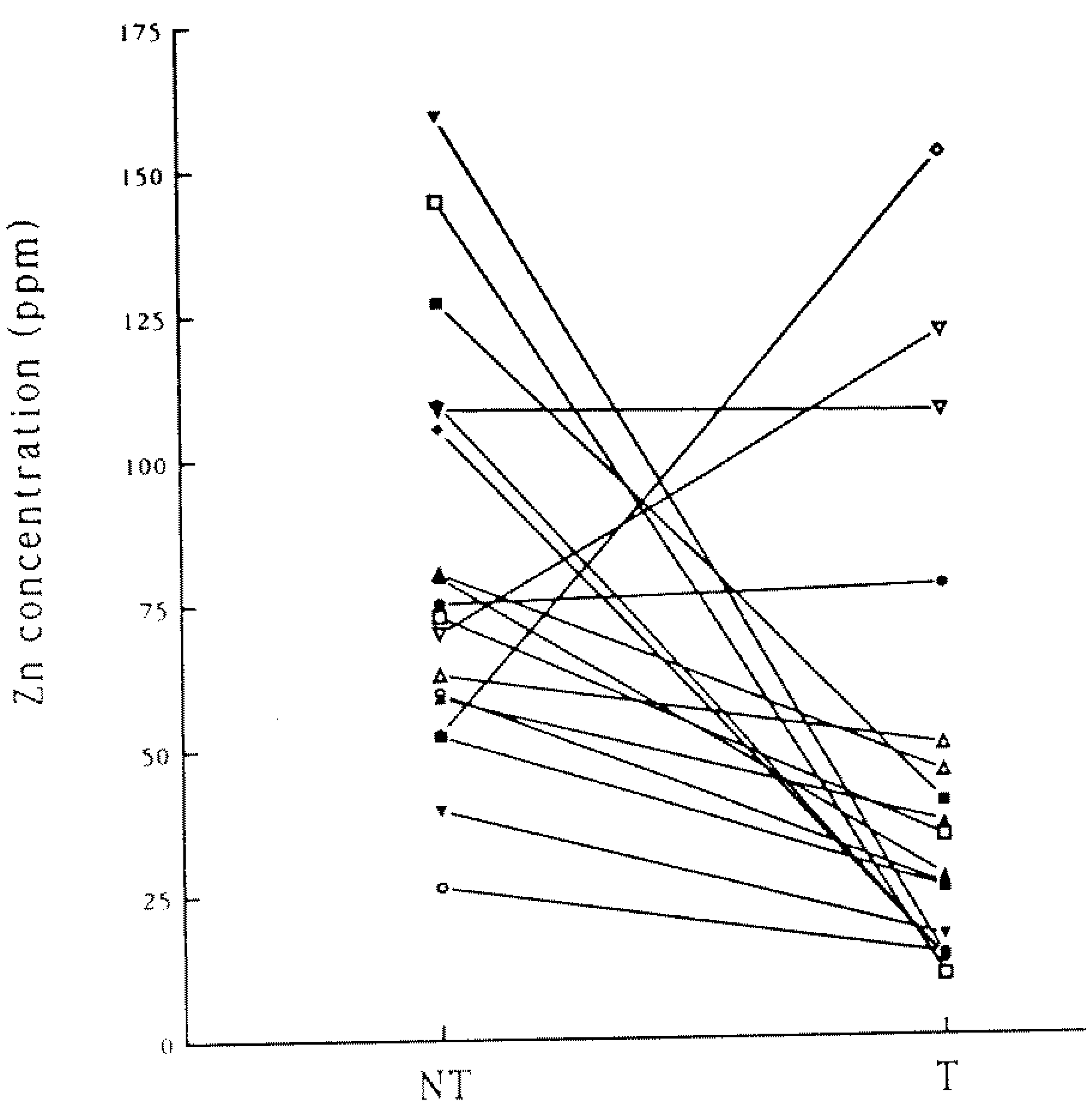

Figure 1. Zinc concentrations in non-tumor (NT) and tumor (T) regions.
Values were presented for each individual and the results were expressed as ppm. The means in NT and T regions were 82.5 and 45.9, respectively. P-value by Student's t-test was 0.019.

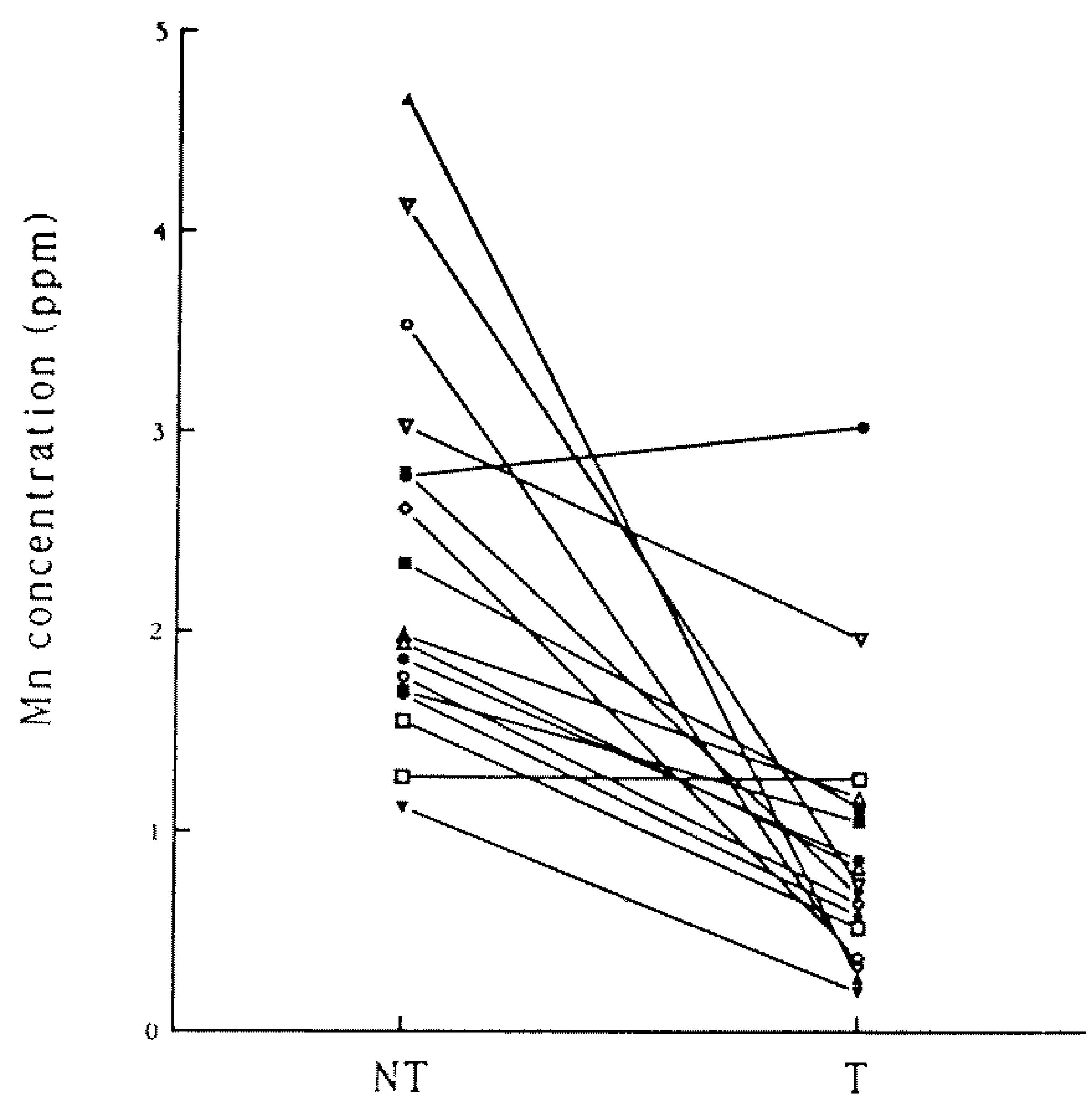

Figure 2. Manganese concentrations in non-tumor (NT) and tumor (T) Regions.
Values were presented for each individual and the results were expressed as ppm. The means in NT and T regions were 2.39 and 0.92, respectively. P-value by Student's t-test was 0.0001.

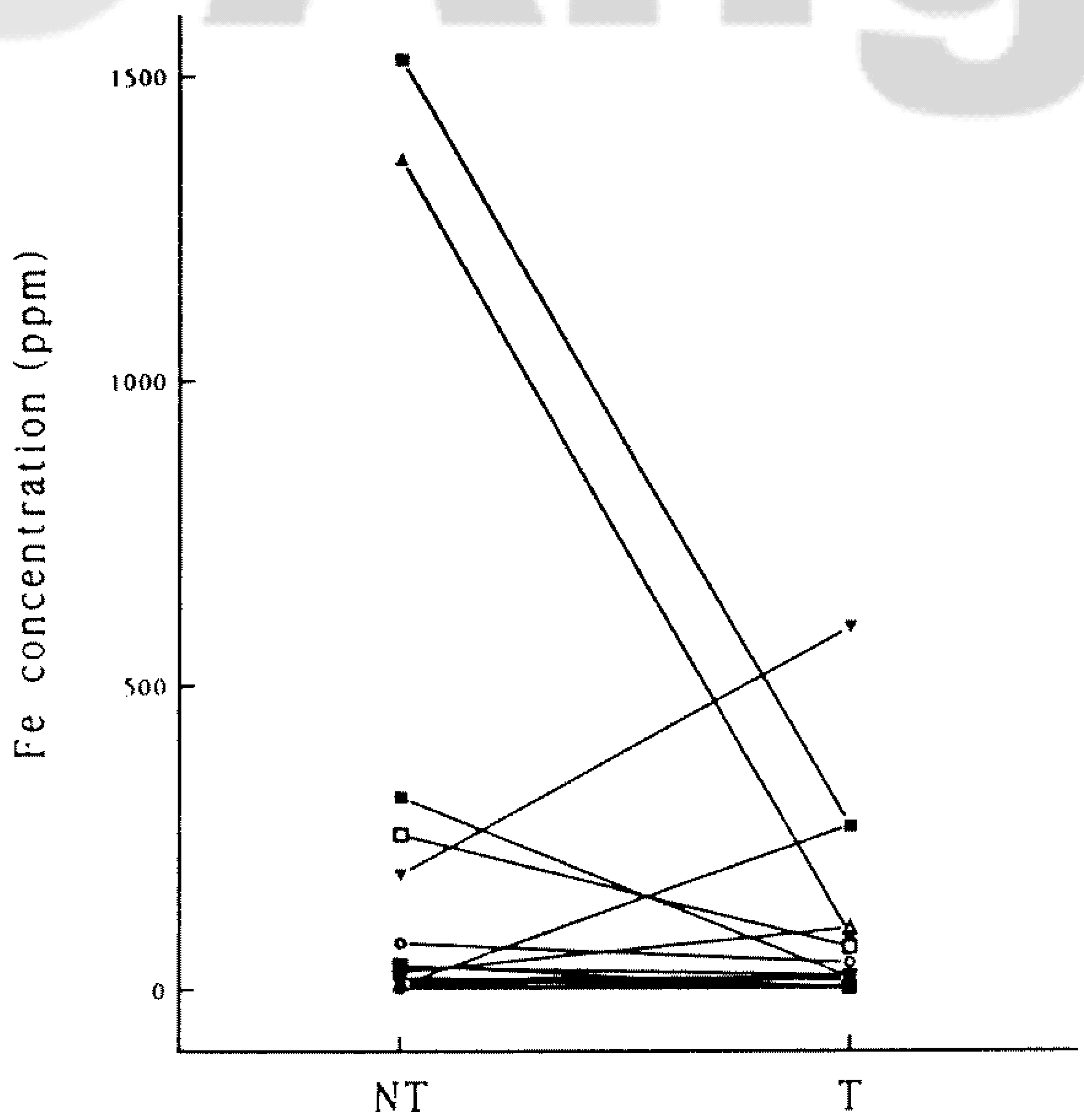

Figure 3. Iron concentrations in non-tumor (NT) and tumor (T) regions.
Values were presented for each individual and the results were expressed as ppm. The means in NT and T regions were 66.2 and 77.4, respectively. P-value by Student's t-test was 0.78.

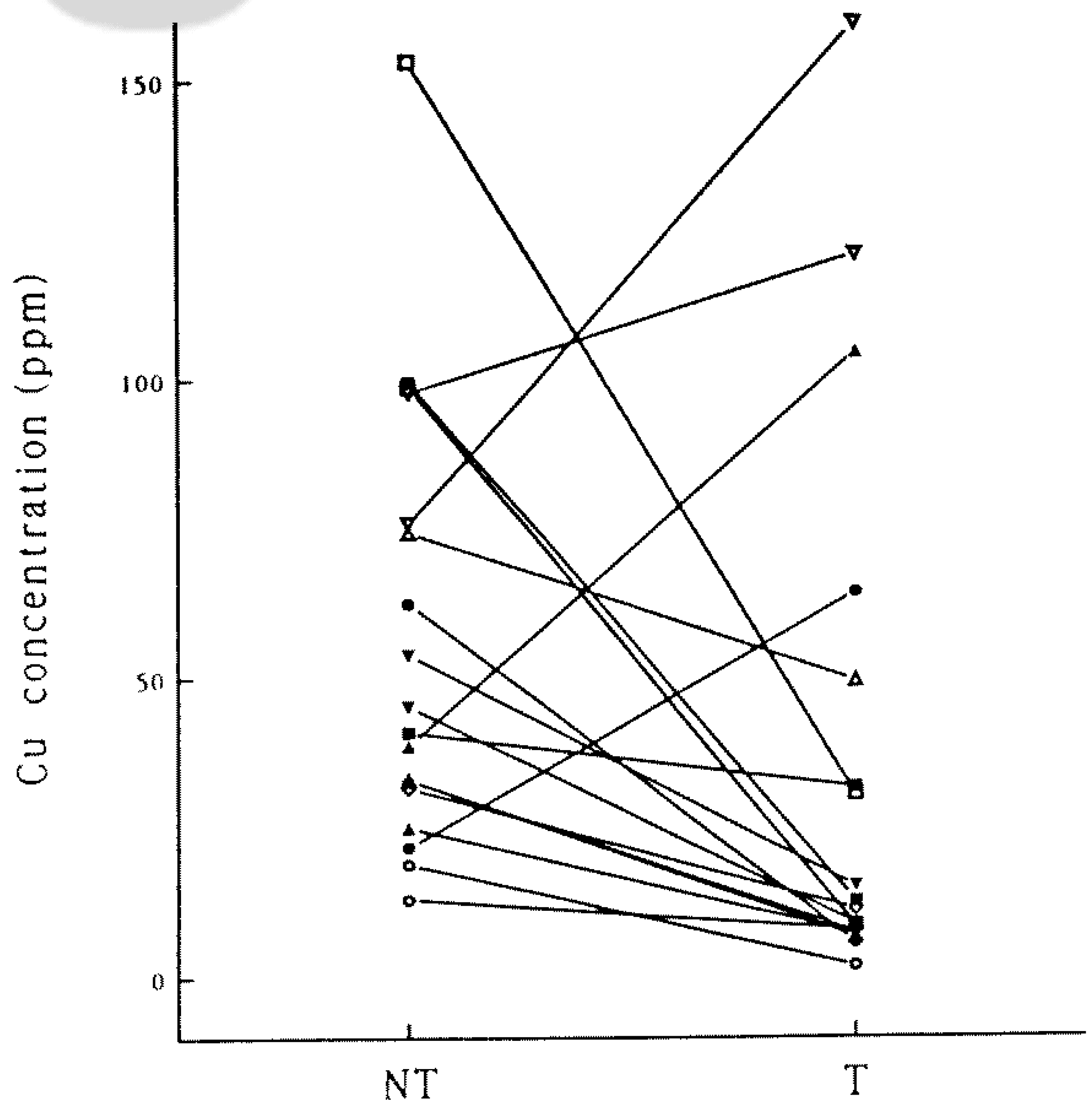

Figure 4. Copper concentrations in non-tumor (NT) and tumor (T) regions.
Values were presented for each individual and the results were expressed as ppm. The means in NT and T regions were 56.5 and 36.2, respectively. P-value by Student's t-test was 0.12.

Table 1. Correlation coefficients between zinc and manganese conecntrations in tumor regions and blood biochemical parameters of HCC patients.

In Blood	In tumor Zn^{+2}	Mn^{+2}
Glucose	0.217	0.327
Cholesterol	0.130	0.100
TG	0.114	0.100
ALP	0.032	0.000
Albumin	0.217	0.100
Calcium	0.105	0.259
AFP	0.321	0.322
AST	0.202	0.045
ALT	0.077	0.000
LDH	0.000	0.164
Tumor Size (cm^3)	0.000	0.158

TG : triacylglycerol; ALP : alkaline phosphatase; AFP : α-fetoprotein; AST : aspartate aminotransferase; ALT : alanine aminotransferase; LDH : lactate dehydrogenase.

圖一至圖四分別爲HCC患者肝腫瘤與非腫瘤組織中可溶於水的鋅、錳、鐵、銅離子之濃度變化情形。由圖一，我們發現鋅離子於腫瘤區域之含量較非腫瘤區域爲低，而且平均濃度爲腫瘤組織45.9 ppm（範圍由10.43-152.08），非腫瘤組織82.5 ppm (26.38-159.27)。利用Student's t-test分析，顯示肝腫瘤組織中鋅離子濃度有意義的低於非腫瘤組織(P <0.05)，此現象與前人的研究結果相符合[11,12,16]。推測其原因可能與腫瘤組織中以鋅爲輔因子的酵素活性下降有關，如lactate dehydrogenase, alcohol dehydrogenase等。

由圖二可得知錳離子濃度於大部分檢體(16/18)之腫瘤組織含量較非腫瘤組織爲低，將其中一個數值超過其他所測得之數值5倍標準差的檢體刪除後統計，其平均濃度爲腫瘤組織0.92 ppm (0.20-3.02)，非腫瘤組織2.39 ppm (1.12-4.66)，顯示肝腫瘤組織中錳離子濃度亦有意義的低於非腫瘤組織(P<0.0005)。由於錳離子爲某些葡萄糖代謝酵素（如glucokinase, pyruvate dehydrogenase）之輔因子，而由文獻中可發現HCC患者之葡萄糖代謝會受到干擾[17]，葡萄糖新生作用所須之酵素活

性降低[18]，這現象是否與錳離子濃度降低有關，尙有待進一步的探討。

鐵離子及銅離子所測得之結果於每個檢體間呈現相當大的個體差異（見圖三，圖四），鐵離子於統計時刪去2個超過5倍標準差之數值後，以n＝16統計結果爲P＝0.78，而銅離子(n＝18)則爲P＝0.12，均不具統計上有意義之差別。故鐵，銅離子在本研究中與HCC並未發現任何相關性，此點與Okuno等人之結果相同[11]。而以MRI測得HCC腫瘤部位有銅離子的增加，推測與膽管阻塞，導致copper-binding protein堆積有關[13,14]，惟此現象可能有個體差異，仍須收集更多病例詳細研究。

表一爲肝腫瘤組織中可溶於水之鋅、錳離子濃度與臨床生化檢查結果之比較，由於相關係數均小於0.5，顯示兩者間並無高度之相關性；此外，腫瘤組織的大小亦與金屬離子之濃度高低無關。

誌　謝

感謝國科會清華大學精密儀器中心柯景南先生在ICP-AES測定上所給予的協助。

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Journal of Food and Drug Analysis. 1994. 2(3)

The Concentrations of Four Metal Ions in Tumor and Non-tumor Regions of Livers from Patients with Hepatocellular Carcinoma

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ABSTRACT

The concentrations of Zn, Mn, Fe, Cu in tumor and non-tumor regions from human hepatocellular carcinoma (HCC) specimens were measured by ICP-AES. Resected liver tissues from eighteen patients with HCC were collected for this study. Lower zinc and manganese ion concentrations were demonstrated in tumor regions (Zn^{+2},$P<0.05$;Mn^{+2},$P<0.0005$). It might indicate lower activities of some glucose metabolic enzymes in HCC tumor tissues, such as lactate dehydrogenase, glucokinase and pyruvate dehydrogenase. No significant differences in the concentrations of iron ($P=0.78$) and copper ($P=0.12$) ions between tumor and non-tumor regions were found.

Key words : hepatocellular carcinoma (HCC), ICP-AES.