行政院國家科學委員會專題研究計畫成果報告

烏腳病盛行地區皮膚癌病例與健康對照之週邊淋巴細胞姊妹染色分體交換頻率與砷甲基化能力及血清微量素之相關性研究

A Study on the Association of Peripheral Lymphocyte Sister Chromatid Exchanges、Chromosome Break and Arsenic Methylation Capability and Serum Micronutrients in Bowen Disease

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一. 中英文摘要

本研究為探討烏腳病高盛行地區皮膚癌與非皮膚癌病患，血液淋巴細胞姊妹染色分體交換頻數與慢性砷暴露指標、營養狀態(血清中α和β-胡蘿蔔素、維生素A、α-維生素E、番茄紅素等微量營養素)和尿液中無機砷之代謝能力(尿液砷代謝物種比例)之間的關係。研究對象乃選擇居住於烏腳病高盛行里，好美、復興、新民三里居民。在80年9月，82年2月與85年9月進行三次健康檢查，經由高醫皮膚科醫師作皮膚科檢查，依照臨床診斷標準判斷。目前本研究中之皮膚癌大都為波文氏症(Bowen's disease)。新發生之波文氏症共21人，另外選取年齡、性別配對非波文氏症者34人為對照組。測定自發性週邊血液淋巴細胞之姊妹染色分體交換頻數。研究對象的尿液利用高效能液相色層分析儀及原子吸收光譜儀進行砷物種(三價砷、五價砷、單甲基砷酸及雙甲基砷酸)分析及定量。血清微量營養素(α-維生素E、α和β-胡蘿蔔素、番茄紅素與維生素A)濃度則利用高效能液相色層分析儀測定。本研究發現皮膚癌病患與健康對象之姊妹染色分體交換頻率隨尿液中無機砷百分比增加而增加，但隨雙甲基砷酸百分比增加而有降低趨勢，未達統計上顯著性。血清中β胡蘿蔔素較低者有顯著偏高的姊妹染色分體交換頻率。其作用機轉仍須作進一步探討。

關鍵詞： 砷、物種分析、微量營養素、姊妹染色分體交換頻數

Abstract

To explore the relationship among cytogenetic damages, sister chromatid exchanges (SCEs) in skin cancer patients and controls and chronic arsenic indices (duration of living in BFD endemic area , duration of drinking artesian well water and cumulative arsenic exposure), serum micronutrients (α and β-carotene, retinol , lycopene and α-tocopherol), and arsenic

methylation capability (ratio of arsenite (AsIII), arsenate (AsV), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA)), residents aged 30 or older were recruited from three arseniasis-hyperendemic villages from January to February 1993. Total 21 cases were diagnosed by experienced dermatologists from Kaohsiung Medical College. In this study all skin cancer were Bowen's disease. In addition, 34 age-sex-matched healthy controls were chosen as study subjects. Spontaneous frequencies of peripheral lymphocytes were compared between cases and controls. Urinary arsenic was examined by high performance liquid chromatography (HPLC) to speciate AsIII, AsV, MMA, DMA and then quantified by atomic absorption spectrophotometry. Serum α and β-carotene, retinol , lycopene and α-tocopherol were tested by HPLC. The spontaneous frequency of sister chromatid exchanges was associated with elevated proportion of urinary inorganic arsenic percentage, and inversely associated with DMA percentage, but not statistically significant. On the other hand, The spontaneous frequency of sister chromatid exchanges was significantly inversely associated with serum β-carotene level, but it needs further studied.

Keywords： • Arsenic Speciation • Liver disease • Micronutrients

二. 計畫緣由與目的

無機砷被公認為人類皮膚癌與肺癌之致癌物(1，2)。砷由於環境自然發生與人類活動而流布於環境中。因為飲用受砷污染之飲用水而發生的健康危害報告不斷地出現在世界各國，其中包括臺灣西南沿海地區(3)，墨西哥北部(4)，智利的安多法加斯大(5)，阿根廷(6)，匈牙利與羅馬尼亞(7)，中國大陸新疆與內蒙古(8，9)，印度的西孟加拉(10)與泰國南部Nakorn Srithammarat省(11)。

烏腳病為臺灣西南沿海地區獨特的週邊血管病變(3)，其發生和飲用當地高含砷深井水有關，兩者之間呈劑量效應關係。烏腳病盛行地區皮膚癌盛行率為千分之10.6，且皮膚癌盛行率與飲用深井水的砷含量呈劑量效應關係(3)。無機砷經消化系統與呼吸系統進入人體後，累積於肺臟、肝臟、腎臟和皮膚等處(12)。長期暴露於無機砷，對許多臟器會發生毒性作用(13)。流行病學研究發現，烏腳病盛行地區比臺灣其他地區有偏高的膀胱癌、腎癌、肺癌、肝癌的死亡率，以臺灣一般族群作比較，其年齡標準死亡比(SMR)男性分別為1100、772、320與170，而女性分別為2009、1119、413、與229(14)。含砷飲用水的長期暴露與皮膚癌、肺癌、肝癌、膀胱癌、腎癌和攝護腺癌死亡率之間呈現顯著的劑量效應關係(15-17)。

在動物實驗中，至今尚無足夠証據証實砷的致癌性(1，2)，但在體外試驗中，砷是哺乳類細胞的一種致染色體變異物(clastogen)。無機砷誘發姊妹染色分體交換頻數(sister chromatid exchange;SCE)增加(18-25)，及染色體異常(20，21，26-27)。另有報告指出砷與紫外線、χ放射線與DNA產生共價結合物質(crosslinking agents)具有共同致染色體變異性(coclastogenicity) (21-27)。免疫缺乏病患皮膚癌和皮膚病變的危險性有增加的現象(28)。無機砷暴露居民的淋巴細胞在體外試驗中，發現淋巴細胞的增生產生減緩的現象(29，30)。三價砷酸鈉與五價砷酸鈉會抑制人類淋巴細胞的增殖(18，23，31)與DNA合成(32)。

自1900-1910年間，烏腳病盛行地區開始使用深井水；而自 1960 年起，該地區部份村里開始裝設自來水。 但是直至1970 年，該地區並未全面換裝自來水設

備。 最近研究發現烏腳病盛行地區三十歲以上居民在終止飲用高砷深井水 約30年後皮膚癌的盛行率仍高達6%，且與飲用水砷含量之間，亦呈劑量效應關係(33) 。此外，又發現無機砷誘發波文氏症病患會增加姊妹染色分體交換頻數且抑制淋巴細胞增殖(34)。

人體攝取無機砷後，其代謝過程相關複雜。無機砷進入體內，五價砷在血液中還原成三價砷(35)後，在肝臟中進行甲基化反應。人體攝取無機砷後，最主要的代謝物為dimethylarsinic acid (DMA)和monomethylarsonic acid (MMA)(36) 。DMA的毒性較低(37)，而且很快地排至尿液中(38)。動物實驗的研究指出，在小白鼠及兔子體中methyl transferase被抑制後，會影響砷的甲基化而造成體內砷滯留的現象(39)。由此結果可能可以證實無機砷的甲基化為哺乳動物中之解毒機轉。尿液中無機砷，單甲基砷酸和雙甲基砷酸所佔比例被用來作為甲基化代謝能力的指標(40)。雖然無機砷甲基化的機轉尚未完全明瞭，但似乎很清楚地知道甲基化是發生在肝臟而且是酵素參與的反應。肝臟功能不良(麩氨基硫枯竭)顯著地改變了尿液中所排泄的甲基化代謝物的比例(41)。所以肝臟疾病也許會影響人體內(41)或大白鼠體內(42)三價砷的甲基化。在最近研究中我們發現慢性B型肝炎表面抗原帶原且肝功能異常者顯著地增加皮膚癌的盛行率(33)。此外，又發現累積砷暴露量越高且砷甲基化代謝能力較差者皮膚癌發生率顯著偏高(43)。

烏腳病盛行地區的居民被發現營養狀況很差(44)。在最近研究中我們亦發現以食用曬乾蕃薯簽為主食的年數作為營養狀況不良的指標，則皮膚癌盛行率和食用曬乾蕃薯簽為主食的年數之間呈現劑量效應關係(33)。動物實驗報告指出，飲食中低methionine, choline和蛋白質的不良營養狀況時會抑制methyl transferase(45)，而造成較低的甲基化能力，因而減弱砷的解毒能力(46)。

β-胡蘿蔔素是一種植物來源的類胡蘿蔔素(carotenoid)，具有維生素A前驅物(retinol)的活性，被認為是對抗癌症的可能預防物質(47，48)。β-胡蘿蔔素及其他的類胡蘿蔔素也許在減少癌症發生扮演著重要角色(48)。維生素A是調節細胞生長與分化的必須營養素(49，50)，試管內實驗發現維生素A可以促進細胞分化(51)抑制細胞轉形(52)與增生(53)。流行病學研究發現攝食蔬菜水果富含之胡蘿蔔素可減少癌症的危險性(54-55)。在最近研究中我們亦發現血清中β-胡蘿蔔素偏低者皮膚癌發生率顯著偏高(43)。其作用機轉至今尚未完全明瞭，可能機制之一為類胡蘿蔔素能增強免疫作用(56)。最近體外試驗研究中，發現飲食攝取較低的胡蘿蔔素會抑制淋巴球的有絲分裂增值(57)。其它維生素，例如維生素E，也被認為是癌症預防物質(58)。

除了砷之外，血清中α和β-胡蘿蔔素、維生素A、α-維生素E、番茄紅素等微量營養素之營養狀況是否會影響烏腳病盛行地區無機砷誘發波文氏症病患增加姊妹染色分體交換頻數?此外，砷代謝能力與無機砷誘發波文氏症病患增加姊妹染色分體交換頻數是否有關?以及血液中微量營養素，砷代謝能力與無機砷誘發波文氏症病患增加姊妹染色分體交換頻數之間是否有交互作用皆需進一步探討。

本研究欲利用本人近年的研究中，由清華大學楊末雄教授協助建立尿液中砷濃度及物種分析方法，進一步探討烏腳病盛行地區皮膚癌與非皮膚癌者週邊淋巴細胞自發與無機砷誘發之姊妹染色分體交換頻數與營養狀態(血清中α和β-胡蘿蔔素、維生素A、α-維生素E、番茄紅素和硒等微量營養素)和尿液中無機砷之代謝物種(三價砷、五價砷、單甲基砷酸、雙甲基砷酸)之間的關係。

本研究的目的有五：

(1)探討烏腳病盛行地區皮膚癌與非皮膚癌者週邊淋巴細胞自發與無機砷誘發之姊妹染色分體交換頻數與慢性砷暴露指標(居住烏腳病盛行地區年數，飲用深井水年數，累積砷暴露)的相關性。

(2)探討烏腳病盛行地區皮膚癌與非皮膚癌者慢性砷暴露指標(居住烏腳病盛行地區年數，飲用深井水年數，累積砷暴露)與尿液中砷物種(三價砷、五價砷、單甲基砷酸、雙甲基砷酸)比例以及血清中微量元素(α和β-胡蘿蔔素、維生素A、α-維生素E、番茄紅素)含量的相關性。

(3)探討烏腳病盛行地區皮膚癌與非皮膚癌者週邊淋巴細胞自發姊妹染色分體交換頻數與尿液中砷代謝物種(三價砷、五價砷、單甲基砷酸、雙甲基砷酸)比例及血清中微量元素(α和β胡蘿蔔素、維生素A、α-維生素E、番茄紅素)含量的相關性。

(4)探討烏腳病盛行地區皮膚癌與非皮膚癌者週邊淋巴細胞自發之姊妹染色分體交換頻數與血清中微量元素(α和β-胡蘿蔔素、維生素A、α-維生素E、番茄紅素)含量及與尿液中砷代謝物種(三價砷、五價砷、單甲基砷酸、雙甲基砷酸)比例間之交互作用。

三. 結果與討論

表1為砷誘發皮膚癌病人和其對照組慢性砷暴露指標的比較。結果顯示皮膚癌病人的累積砷暴露、飲用深井水年數與居住於烏腳病盛行地區年數比對照組顯著偏高。

表2為砷誘發皮膚癌病人和其對照組年齡、飲食及各種肝功能指標的比較。結果顯示皮膚癌病人和其對照組之年齡、穀氨酸草醋酸氨基轉移酶、穀氨酸丙酮酸氨基轉移酶與胎兒蛋白並無顯著差異，但皮膚癌病人攝食曬乾蕃薯纖年數顯著比對照組偏高。

表1 砷誘發皮膚癌病人和其對照組慢性砷暴露指標的比較

變項	病例組	對照組
	(平均值±標準差)	(平均值±標準差)
累積砷暴露 (ppm-years)	21.627±2.090*	17.356±1.412*
飲用深井水年數 (years)	29.400±2.589*	23.882±1.699*
居住於烏腳病盛行地區年數 (years)	54.600±1.397*	49.794±2.004*
平均砷濃度 (ppm)	0.733±0.025	0.716±0.345

表2 砷誘發皮膚癌病人和其對照組年齡、飲食及各種肝功能指標的比較

變項	病例組	對照組
	(平均值±標準差)	(平均值±標準差)
年齡	59.067±1.343	58.112±1.488
攝食曬乾蕃薯纖年數	22.000±2.195*	14.588±1.851*
穀氨酸草醋酸氨基轉移酶	28.933±3.576	27.912±1.629
穀氨酸丙酮酸氨基轉移酶	23.067±3.959	25.882±2.502
胎兒蛋白	2.649±0.366	2.459±0.654

表3為砷誘發皮膚癌病人和其對照組尿液中各種砷代謝物種的比較。結果顯示皮膚癌病人和其對照組尿液中三價砷酸、雙甲基砷酸、單甲基砷酸、五價砷酸與總砷量並無顯著差異。

表3 砷誘發皮膚癌病人和其對照組尿中各種砷代謝物種的比較

變項	病例組	對照組
	(平均值±標準差)	(平均值±標準差)
三價砷酸	1.509±0.346	2.301±0.405
雙甲基砷酸	70.548±16.517	71.011±10.810
單甲基砷酸	15.818±3.098	15.683±2.224
五價砷酸	6.363±1.214	4.464±0.826
總砷量	94.238±20.301	93.460±12.692

表4為砷誘發皮膚癌病人和其對照組各種砷代謝物種百分比與一級、二級指標的比較。結果顯示皮膚癌病人和其對照組尿液中無基砷酸百分比、雙甲基砷酸百分比、單甲基砷酸百分比、一級與二級指標並無顯著差異。

表4 砷誘發皮膚癌病人和其對照組各種砷代謝物種百分比與一級、二級指標的比較

變項	病例組 (平均值±標準差)	對照組 (平均值±標準差)
無機砷百分比	21.991±8.266	21.830±5.705
雙甲基砷酸百分比	71.910±1.661	72.800±2.501
單甲基砷酸百分比	18.098±1.550	18.846±2.077
一級指標	2.315±0.357	2.850±0.936
二級指標	4.420±0.470	6.593±1.116

表5為砷誘發皮膚癌病人和其對照組血清中微量營養素的比較。結果顯示皮膚癌病人血清中蕃茄紅素與β-胡蘿蔔素比對照組顯著偏低。

表5 砷誘發皮膚癌病人和其對照組血清中微量營養素的比較

變項	病例組 (平均值±標準差)	對照組 (平均值±標準差)
維生素A	0.622±0.060	0.632±0.049
維生素E	5.720±0.818	5.911±0.848
蕃茄紅素	0.890±0.252**	3.653±0.778**
α-胡蘿蔔素	0.983±0.513*	3.156±1.035*
β-胡蘿蔔素	0.641±0.506**	5.788±1.941**

表6是烏腳病盛行地區姊妹染色分體交換頻率在調整年齡、性別、平均砷濃度、β-胡蘿蔔素、攝食曬乾蕃薯纖年數、無機砷百分比的複迴歸分析。結果顯示姊妹染色分體交換頻率在調整其他危險因子後，隨無機砷百分比增加而增加，但隨β-胡蘿蔔素增加而顯著減少。

表6 烏腳病盛行地區姊妹染色分體交換頻率在調整年齡、性別、平均砷濃度、β-胡蘿蔔素、攝食曬乾蕃薯纖年數、無機砷百分比的複迴歸分析

變項	母數估計（標準誤差）	P值
年齡	0.852(0.792)	0.292
性別	14.583(12.138)	0.240
平均砷濃度(ppm)		
0.62-0.69 vs. ≤0.62	25.905(14.507)	0.086*
0.69-0.81 vs. ≤0.62	-4.351(14.417)	0.765
>0.81 vs. ≤0.62	6.471(16.472)	0.698
β-胡蘿蔔素		
0.14-0.33 vs. ≤0.14	-28.793(11.882)	0.023*
>0.33 vs. ≤0.14	-27.113(11.520)	0.026*
攝食曬乾蕃薯纖年數	-8.038(10.588)	0.455
無機砷百分比	0.100(0.160)	0.538

表7烏腳病盛行地區姊妹染色分體交換頻率在調整年齡、性別、平均砷濃度、β-胡蘿蔔素、攝食曬乾蕃薯纖年數、雙甲基砷酸百分比的複迴歸分析。結果顯示姊妹染色分體交換頻率在調整其他危險因子後，隨雙甲基砷酸百分比增加而降低，亦隨β-胡蘿蔔素增加而顯著減少。

表7 烏腳病盛行地區姊妹染色分體交換頻率在調整年齡、性別、平均砷濃度、β-胡蘿蔔素、攝食曬乾蕃薯纖年數、雙甲基砷酸百分比的複迴歸分析

變項	母數估計（標準誤差）	P值
年齡	0.989(0.876)	0.271
性別	16.421(14.101)	0.257
平均砷濃度(ppm)		
0.62-0.69 vs. ≤0.62	29.786(16.506)	0.085
0.69-0.81 vs. ≤0.62	-5.584(16.434)	0.737
>0.81 vs. ≤0.62	12.149(18.624)	0.521
β-胡蘿蔔素		
0.14-0.33 vs. ≤0.14	-35.368(14.016)	0.019*
>0.33 vs. ≤0.14	-31.001(14.383)	0.042*
攝食曬乾蕃薯纖年數	-5.932(13.284)	0.660
雙甲基砷酸百分比	-0.582(0.576)	0.323

四. 計畫成果自評

本研究成果發現烏腳病盛行地區已停止飲用深井水約三十年，而皮膚癌病患與健康對象之姊妹染色分體交換頻率隨尿液中無機砷百分比增加而增加，但隨雙甲基砷酸百分比增加而有降低趨勢，未達統計上顯著性。此顯示砷代謝能力較差者姊妹染色分體交換頻率可能偏高。本研究亦發現血清中β胡蘿蔔素較低者有顯著偏高的姊妹染色分體交換頻率。由此研究結果應建議烏腳病盛行地區居民多攝取蔬菜水果，將可減少姊妹染色分體交換頻率，而避免癌症之發生。

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