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Protective Effects of Omega-3 Fatty Acid on Traumatic or Ischemic Brain Injury

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Because of extensive use of motorcycles as the traffic vehicle in Taiwan, traumatic brain injuries (TBI) has been particularly prevalent not only in car or sports accidents but also in young individuals encountering motorcycle accidents. Traumatic brain injury (TBI) causes tissue damage by primary and secondary injuries to the neural tissue. Primary injury is due to initial mechanical trauma resulting in physical disruption of vessels, neurons and their axons. Secondary injury is due to a series of complex events at the subcellular level following the primary impact and causes the death of additional cells at the peripheral zone of the initial damage. Secondary injury is considered to be the main target of medical treatment. Docosahexaenoic acid, a principle omega-3 polyunsaturated fatty acids (PUFA) of fish oils or marine algae, is of particular interest as it is found in the cellular membranes of most human tissues and is converted to protectin D1 (PD1) or neuroprotectin D1 (NPD1) in neural tissue, which exhibits antiinflammatory and proresolving bioactions. It has been known for quite a while that omega-3 fatty acids including DHA and eicosapentaenoic acid (EPA) participate in cell functions. By minimizing activation of toxic pathways and to enhance activity of endogenous neuroprotective mechanisms, ω-3 polyunsaturated fatty acids (n-3 PUFAs such as DHA and EPA) show the ability in neuroprotection. In addition, collective evidence suggests that antioxidant, anti-inflammatory, and anti-apoptotic properties of DHA and EPA (perhaps through their metabolite NPD1) may act in combination to contribute to their neuroprotective effect. In this review, we summarize the evidence available to date that indicates that ω-3 PUFAs are neuroprotective in brain injuries caused by trauma or ischemia. (Tungs' Med J 2009; 3: 57-66)

Key words: Brain injury, trauma, ischemia, Omega-3 fatty acid, DHA, EPA

INTRODUCTION

Traumatic brain injury (TBI) is one of the leading causes of death and disability worldwide[1-2]. Globally, the incidence of TBI is generally reported as approximately 200 in 100,000 individuals with a mortality rate of about 20 per 100,000[2]. Accidents involving automobile (and /or motorcycles in Taiwan) are reported to be the major cause of TBI, while falls and assaults also contribute greatly to the number of traumatically injured patients[1-2]. In the United Kingdom, 200-300 per 100,000 people are hospitalized each year due to TBI[3] and the incidence is even higher in areas such as Southern Australia and South

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Africa[4-5] In the United States, TBI is associated with the death of approximately 51,000 people each year and causes long-term disability that affects an estimated 70,000 to 90,000 individuals annually[6-7]. TBI is particularly prevalent in young individuals[8]. In Europe, at least 11.5 million people suffer from disabilities related to a TBI[9].

Because of the rapid industrial and economic growth, Taiwan and other developing countries have faced an enormous increase in the number of motorcycles, which has subsequently caused a rapid increase of the motorcycle-related traumatic brain injuries (TBI). Given the global prevalence and enormous societal and economic costs of TBI, it is imperative to understand the mechanisms that contribute to dysfunction and death following trauma, at the same time we may devise appropriate therapeutic strategies to improve patient outcome.

Pathophysiology of Traumatic Brain Injury

The pathophysiology of TBI consists of two main phases: a primary (mechanical) phase of damage, and a secondary (delayed) damage phase. Primary damage occurs at the moment of insult, and includes contusion and laceration, diffuse axonal injury and intracranial hemorrhage[10-11]. Secondary damage includes brain damage due to altered neurochemical mechanisms, activation of degradative enzymes, swelling (edema) and ischemia. Evidence of secondary injury, like apoptotic as well as necrotic cell death of neurons was detected in the brains of rats subjected to experimental TBI (Fig. 1). Ischemia has been suggested as one of the most important mechanisms underlying secondary brain damage following TBI, especially in severely injured patients [12-14]. In fact, approximately 90% of traumatically injured patients who die demonstrate ischemia on histopathological examination of brain tissue[15].

Excitotoxicity, peroxynitrite (nitric oxide and free radicals) leading to secondary injury following TBI

Glutamate and aspartate, two excitatory neurotransmitters, are released in an uncontrolled manner in ischemic and traumatic areas. Glutamate activates the ionophoric NMDA and AMPA receptors, which leads to influx of calcium, sodium and water into cells of the injured region. Elevated intracellular calcium causes an increase in cellular oxidative stress that contributes to cell damage. Intracellular calcium also activates various enzymes such as lipases, proteases and endonucleases that may damage DNA, cell proteins and lipids thus leading to cellular death.Activation of AMPA and aspartate receptors that cause exmembrane depolarization was shown to contribute to excitotoxic damage and cell death[16].

Nitric oxide (NO•) produced by of enzymatic pathway of nitric oxide synthase (NOS) is a small diffusible molecule it could exert a paracrine, vasodilatatory effect on surrounding blood vessels [17-20]. Overproduction of NO has been demonstrated after traumatic injury [21]. NO can inhibit platelet aggregation, induce leukocyte infiltration. Following TBI, excessive NO• can react with superoxide anion $O^{2\,\bullet-}$ to produce peroxynitrite ($ONOO^-$), which is highly toxic to neuronal proteins, membrane lipids and DNA [22-23]. NOS itself produces O2 •-, but levels are continually regulated by endogenous free radical scavengers such as SOD. After trauma, however, when there is overactivation of NOS and additional mechanisms producing O2 •-, endogenous scavengers like SOD may become saturated, allowing high levels of ONOO- to form, ultimately leading to neuronal damage (Fig.1).

Prolonged elevations of intracellular calcium also results in formation of superoxide anion radicals by the respiratory chain, as well as by cytosolic enzymes, such as xanthine oxidase. This leads to an increase in reactive oxygen species (ROS) and oxidative stress [24]. The brain also contains high levels of transition metals, such as iron, copper and manganese. These redox-active metals are capable of catalyzing the production of highly toxic radicals via the metal-mediated Haber–Weiss reaction. Following TBI, an increase in lipid peroxidation, production of peroxynitrite, and impairment of the endogenous antioxidant system have been demonstrated in rats. Similarly, a sustained decrease in the total antioxidant reserve including ascorbate and glutathione has been observed in the cerebrospinal fluid in infants and children after severe TBI [25].

TBI causes delayed neuronal death by mechanism of apoptosis and autophagy

Traumatic brain injury (TBI) causes tissue damage by primary and secondary injuries to the neural tissue. Primary injury due to initial mechanical trauma results in physical disruption of vessels, neurons and their axons (axotomy and necrosis). Whether the

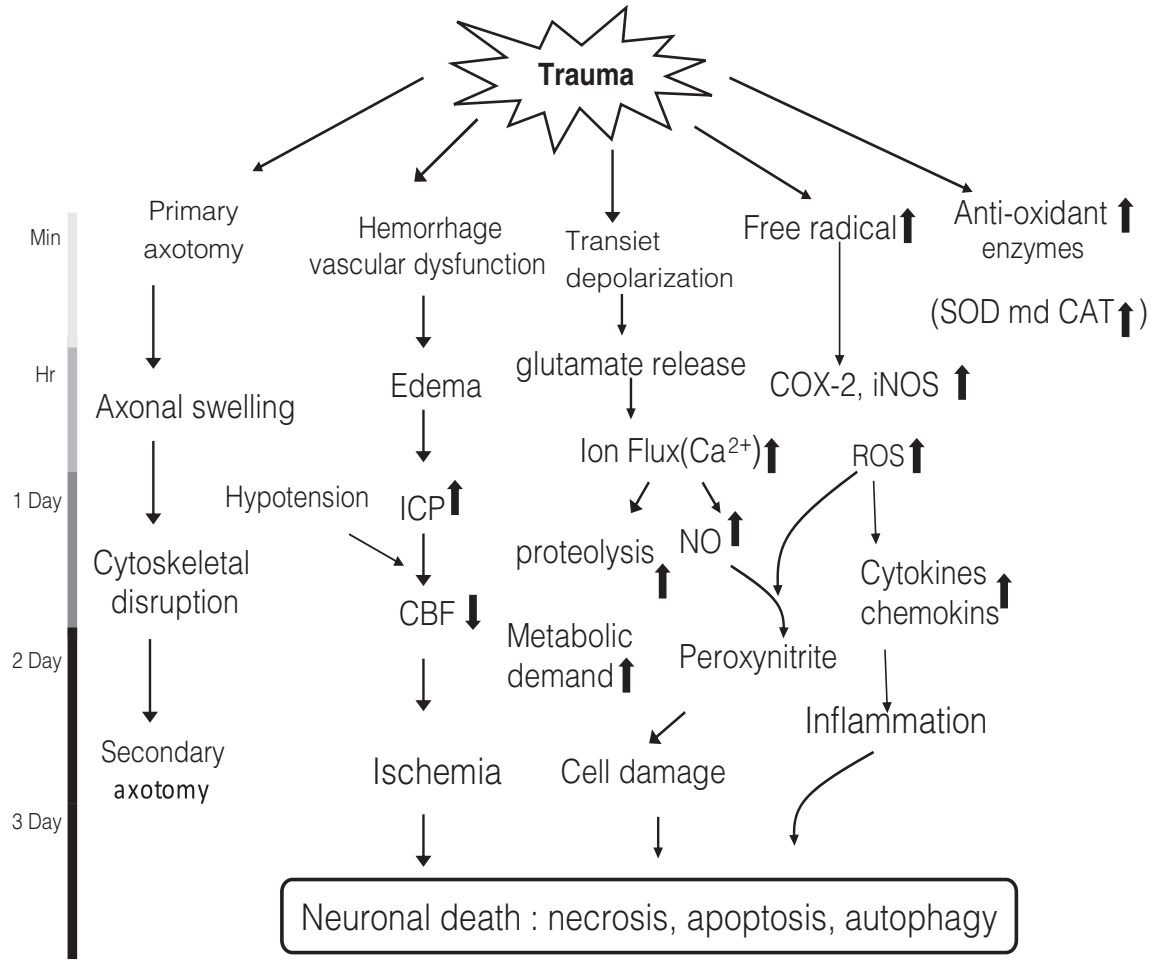

Fig. 1 Pathophysiological events following traumatic or ischemic brain injury.
Abbreviations: ICP: intracranial pressure; CBF: cerebral blood flow; ROS:Reactive oxygen species; SOD: superoxide dismutase; CAT: catalase; MDA: malondialdehyde; iNOS: inducible nitric oxide synthase; COX: cyclooxygenase

processes initiated at the moment of injury convert into final irreversible brain damage depends on such secondary injury cascades. The delayed nature of these events provides an opportunity for therapeutic intervention aimed at either minimising the process of progressive damage or enhancing the processes that promote recovery the neurochemical sequelae during secondary injury are complex, involving excess release of excitatory amino acids, particularly glutamate, disruption of ionic homeostasis due to Na^+ and Ca^{2+} influx and generation of toxic free radicals, ultimately leading to cell death by necrosis, apoptosis and autophagy (Fig.1).

Apoptosis may be responsible for up to 50% of cellular death in ischemia[26], and trauma[27]. Both extra- and intracellular signals that have been reported to initiate this process were identified after brain injury. Whereas intracellular signals for apoptosis are triggered by mitochondrial dysfunction, the extra cellular signals involve the activation of the TNF super family receptors that activate death domains and recruit caspases[28]. Autophagy is a homeostatic process for recycling of proteins and organelle, mediating clearance of long lived, oxidized, or aggregated proteins and organelles[29-31], while aberrant autophagy resulted in neurite degeneration and neuronal cell death, as shown in several in vitro and in vivo systems[32-35]. Autophagy occurs after both

experimental and clinical TBI[36]. Autophagy acts as a double-edged sword after TBI, as during long term of repair after TBI, autophagy protects nerve cells injured slightly from death by eliminating noxious agents such as injured cellular organ and excitatory amino acids, and in the meanwhile is also responsible for unrecoverable nerve cells death[37].

Inflammatory mediators leading to secondary injury

The presence of inflammatory cells in the ischemic and around the traumatic region may increase cellular damage. Inflammatory cytokines such as TNFa, IL-1 and IL-6 appear to be robustly activated and secreted as early as 1 hour after the ischemic and traumatic insults[38-42]. These cytokines may induce an inflammatory reaction and also act as chemoatractants to leukocytes. Inhibition of the inflammatory response during its acute phase was shown to lead to better recovery in models of cerebral ischemia and trauma.

Features of Omega-3 Fatty Acids

There are twelve essential fatty acids (EFAs) that are subdivided into two structural categories depending on the saturation state of the molecule. The designation omega-3 (ω-3) or omega-6 (ω-6), respectively, indicates if the third or sixth carbon from the methyl terminus is unsaturated. The ω-3 andω-6 fatty acids are derived from the dietary intake of the 18-carbon precursors a-linolenic acid and linoleic acid, respectively. The ω-3 fatty acids include: a-linolenic acid (18:3 ALA), stearidonic acid (18:4), eicosatetraenoic acid (20:4), eicosapentenoic acid (20:5 EPA), docosapentenoic acid (22:5), and docosaehexanoic acid (22:6 DHA) (Fig. 2).

Dietary supply of long-chain ω-3 and ω-6 fatty acids is essential because mammals are incapable of synthesizing fatty acids with a double bond past the D-9 position. Fish oil is the major source of the omega-3 fatty acids eicosapentaenoic acid (EPA; 20:5*n*-3) and docosahexaenoic acid (DHA; 22:6*n*-3). In the absence of fish oil.

α-linolenic acid is the precursor to EPA and DHA. DHA and EPA, both are ω-3 or n-3 fatty acids, are essential polyunsaturated fatty acids (PUFA) for the brain. They are obtained either directly from

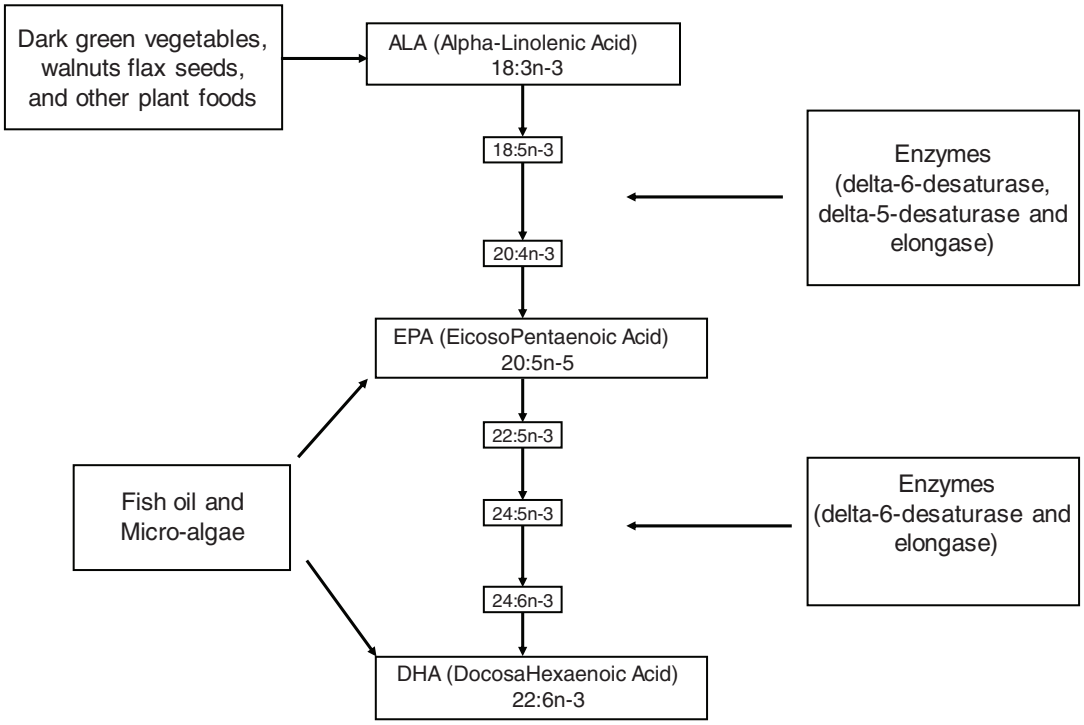

Fig. 2 Biosynthesis of Omega-3 Fatty Acids.

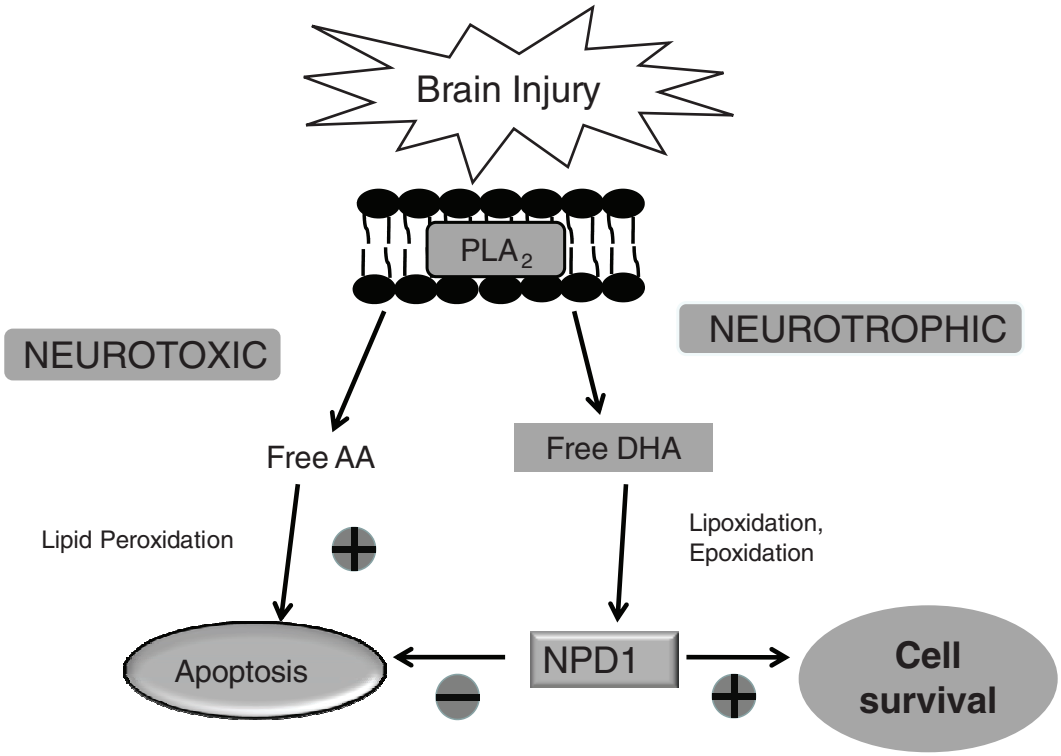

Fig. 3 Free arachidonic acids (AA; 20:4*n*-6) and DHA (22:6*n*-3) are released from membrane phospholipids through the action of phospholipases inresponse to stimulation. AA is synthesized from dietary sources of linoleic acid, whereas the *n*-3 PUFA (including DHA) are synthesized from *α*-linoleic acid. The balance of products of AA and DHA will result is either neurotrophic (promotong survival) or neurotoxic (promotong apoptosis). Therapeutic strategies targeted to enhance the survival and inhibit apoptosis will shift the balance in favor of neuroprotection in brain injury in which a neuroinflammatory component is involved.
Abbreviations : PLA2: phospholipase A2; AA: Arachidonic acid; NPD1: neuroprotectin D1; ⊕: enhancement; ⊖: inhibition

the diet or synthesized from its main dietary ω-3 precursor, ALA, in the liver[43-44]. In neural membranes, EPA and DHA not only affect their physicochemical properties such as membrane fluidity, permeability, and viscosity, especially in neuronal synapses, but also modulate neurotransmission[45-49], gene expression[50-51], activities of enzymes and receptors, ion channels, and immunity[52-54]. Astrocytes can convert some ALA to DHA[55]. Both types of fatty acids are incorporated as structural components of neural membrane glycerophospholipids and are substrates for generating lipid mediators.

Ischemia or trauma can lead to rapid activation of phospholipase A2 resulting in free arachidonic acid (AA) and DHA accumulation (Fig.3). These fatty acids are released from membrane phospholipids in response to stimulation (e.g. neurotransmitters, cytokines, seizures, ischemia, trauma, etc.)[56-57]. This response tells us that phospholipases are regulatory gate keeper in the initiation of the eicosanoid and docosanoid pathways under both physiologic and pathologic conditions. The metabolic utilization of ω-3 fatty acids differs from that of ω-6 fatty acid metabolism. Oxygenation of ω-6 fatty acids generates proinflammatory mediators such as prostaglandins and leukotrienes whereas ω-3 fatty acids generate anti-inflammatory lipid mediators such as resolvins, protectins and neuroprotectin D1 (NPD1)[58-59]. In fact, the positive effects of DHA have been attributed to its ability to antagonize the production of AA and its derivatives, the eicosanoids[60-61] (Fig.3).

Neuroprotective Effect of Omega-3 Fatty Acids on TBI

Anti-oxidant and anti-apoptotic Effects of Omega-3

Table 1. Neuroprotective Effect of Omega-3 on Traumatic or Ischemic Brain Injury

Type of omega-3	Neurological disorder	Experiment model	Effect	Mechanism	References
EPA and DHA	Cerebral ischemia reperfusion	SD rats	neuroprotective effects	MDA levels ↓ scavenging ROS produced during oxidative stress a significant increase for CAT activities prevent apoptosis	67
Fish n-3 EFA	Cerebral ischemia Reperfusion	SD rats	protective effect	SOD activities and MDA levels ↓ the oxidative status and apoptotic ↓ CAT activities and NO levels ↓	62
EPA and DHA	Traumatic brain injury	SD rats	normalizing levels of molecular systems associated with energy homeostasis	normalized the levels of uMtCK after lesion restored the correlation between Sir2 and AMPK or p-AMPK	25
DHA	Brain ischemia	Neuro 2A cell line	promotes neuronal survival	inhibition of apoptosis by polyunsaturates is PS-Dependent facilitates Akt translocation	60
EPA and DHA	Platelet aggregation occurs in cerebral arterioles	mice	significantly slows platelet aggregation	produces a moderate reduction in serum TxB2 level	79
Omega-3 fatty acids	Traumatic brain injury	rat	maintain neuronal function and plasticity counteracted learning disability	normalized levels of BDNF associated synapsin I and CREB reduced oxidative damage	25

Abbreviations:
ROS: reactive oxygen species
SOD: superoxide dismutase
AMPK: AMP-activated protein kinase
CREB: cyclic AMP-response-element-binding protein
CAT: catalase
TxB2: thromboxane B2
MDA: malondialdehyde
BDNF: brain-derived neurotrophic factor

Fatty Acids

The omega-3 fatty acids supplementation caused inhibition of lipid peroxidation, attenuation of the depletion of GSH level, prevention of decline in the activities of GSH-PX and CAT, reduction of locomotor hyperactivity, and decrease in the hippocampal CA1 neuronal loss depending on the ischemic injury[62]. Evidence showing a decrease in RNA/DNA oxidation suggests that the neuroprotective effect of omega-3 fatty acids involved a significant antioxidant function[63]. An increase in antioxidant enzyme capacity of the brain following I/R is important for the primary endogenous defense against free radical-induced injury[64]. The omega-3 fatty acids may have made the brains more susceptible to lipid peroxidation, thus inducing the antioxidant enzymes and reducing NO levels in ischemic events that lead to a beneficial effect[65-66]. The n-3 PUFA also has the ability to decrease MDA levels and SOD activities and increase CAT activity after TBI[67].

The omega-3 fatty acids increases neuronal survival by preventing cytoskeleton perturbations, caspase activation and apoptosis[68-70]. DHA is the precursor of neuroprotectin D1 that was shown to confer resistance to oxidative stress-induced apoptosis[71]. In addition, DHA promote phosphatidylserine accumulation in cell membranes of Neuron cells, thereby enhancing Raf-1 and Akt translocation/activation as well as the associated survival pathways[60, 72]. By preserving the capacity of neurons to phosphorylate ERK1/2, DHA has proven its involvement in maintaining a sufficient rate of phosphorylated active pro-

teins, thereby promoting essential survival pathways in neurons.[73]

Protective Effect of Omega-3 Fatty Acids on Neurons

DHA can promote cell survival by assisting in the maintenance of basal Akt activity under an adverse condition. It facilitate translocation and phosphorylation of Akt, highly enriched in neuronal membranes, suppressing caspase-3 activation and cell death, thus promoting neuronal survival[60,74]. Another neuron protection mechanism of ω-3 PUFA is a reduction in glutamate-induced toxicity[75]. The omega-3 fatty acids may block depolarization-induced increased glutamate efflux and N-methyl-D-aspartate receptor activation partly via inhibition of voltage-sensitive Na+ and Ca2+ channels[63, 76].

Effects of Omega-3 Fatty Acids on Glia cells

The omega-3 PUFAs (DHA) treatment not only increases the number of oligodendrocytes , but also causes the recruitment of macrophage and microglial and significantly lower the levels of immunoreactivity after injury[60]. The omega-3 fatty acids show anti-inflammatory actions through multiple mechanisms. AA and DHA enhance acetylcholine release in the brain and acetylcholine suppress inflammation by inhibiting tumor necrosis factor-apha production[77]. DHA also inhibits COX activity and the formation of proinflammatory eicosanoids and cytokines[63]. EPA inhibits the release of the proinflammatory cytokines interleukin-1 beta (IL-1) and TNF-apha[78].

SUMMARY

The pathophysiology of TBI or ischemia is usually described in terms of primary and secondary events. Whether primary injury is converted into final irreversible brain damage depends on secondary injury cascades. The secondary injury provides an opportunity for therapeutic intervention aimed at either minimizing the process of progressive damage or enhancing the processes that promote recovery the neurochemical sequelae. DHA, a major component of fish oil and marine algae, is most highly concentrated in the cellular membranes of photoreceptors, brain, and synapses. Brain has a convenient and readily accessible supply of DHA that, through highly regulated, phospholipase-mediated exoprotease activities, liberates membrane-bound DHA to serve in neuroprotective and cell fate-signaling roles. The beneficial neurophysiological actions of DHA occur in part through its direct maintenance of neuronal plasma membrane fluidity and functional integrity, and in part through the generation of docosanoids. DHA is also a primary lipid peroxidation target in oxidative injury, and reduced free DHA levels are associated with neurological dysfunction. The nature of the switch from neuroprotective to membrane disruptive and oxidative roles for DHA, such as the generation of NPD1 vs. neuroprostanes, is under intense research.

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OMEGA-3 脂肪酸對於腦創傷與腦缺血之保護作用

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在台灣，機車為普遍使用的交通工具，除了車禍、運動傷害等因素，青年人口發生機車事故致使外傷性腦損傷的現象也甚為頻繁。外傷性腦損傷因初級與次級損傷傷及神經組織，造成腦組織損害，初級損傷是由於初期機械性外傷造成血管、神經，及軸突破裂；次級損傷則因細胞間與細胞內一連串伴隨初級損傷而來的效應，致使鄰近受傷區域其他細胞受損，而次級損傷也正是臨床治療的主要範疇。DHA 為 omega-3（又稱 n-3）不飽和脂肪酸主要成分，存在於魚油及海藻中，研究發現大部分人體組織中的細胞膜有 DHA 的存在，使得此一議題頗為熱門，而在神經組織中 DHA 則轉換為 protectin D1（PD1）或 neuroprotectin D（NPD1），具有抗發炎及神經保護之作用，到目前為止研究發現，omega-3 不飽和脂肪酸包含 DHA 及 EPA，參與細胞的運作，藉由一方面抑制細胞毒性之活化以及另一方面助長內在神經保護機制，發揮 omega-3 不飽和脂肪酸（如 DHA、EPA）神經保護之功效，另外，相關研究指出 DHA 及 EPA 具有抗氧化、抗發炎、與抗細胞凋亡的成分（可能與其產生 NPD1 代謝物有關），其二者相結合之下能發揮保護神經之功效。在本文中，我們回顧並總結目前為止相關之研究，證明 omega-3 不飽和脂肪酸對於腦創傷及腦缺血有神經保護之功效。

（童綜合醫誌 2009; 3: 57-66）

關鍵詞：腦損傷、創傷、局部性缺血、Omega-3 不飽和脂肪酸、DHA、EPA

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正常妊娠及子癲前症孕婦胎盤組織中滋養層細胞其凋亡與增殖表現的研究

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前言： 子癲前症（preeclampsia）是一種在妊娠20週以後發生高血壓、蛋白尿及水腫的疾病，它的確實病理機轉目前未明，而胎盤就是一個重要因素。胎盤中的滋養層細胞（trophoblastic cell）凋亡（apoptosis）與增殖與子癲前症是否有關，目前研究的資料仍不多，本篇研究主要目的在比較子癲前症與正常懷孕胎盤，其滋養層細胞凋亡及增殖的表現是否有所不同，以期更了解及解釋子癲前症的病理機轉。

研究材料與方法： 胎盤組織是從7位正常足月妊娠與7位子癲前症個案取出，她們皆是接受剖腹生產，細胞凋亡的研究是利用TUNEL（TdT-mediated dUTP-biotin nick end labeling，脫氧核糖核苷酸末端轉移媒酶介導的缺口末端標記法）方法，在評估細胞增殖的方法是利用免疫組織化學染色法，測出Ki-67、Bcl-2, 在滋養層細胞的免疫組織化學表現。結果評估方式則是利用下列兩項：一為positive index rate（%）代表染色有陽性反應者占所有細胞的比例。二為semiquantitative immunohistochemical Remmele score（IRS）。皆利用光學顯微鏡來做為計數工具，獲得平均值 ± 標準差。比較子癲前症與正常者胎盤滋養層細胞在凋亡與增殖表現的不同。

研究結果： 在子癲前症胎盤滋養層細胞發生細胞凋亡的平均值是0.37 ± 0.04%，高於正常胎盤是0.18 ± 0.05%。Ki-67的表現在子癲前症滋養層細胞發生的平均值是4.60± 0.20%，高於正常胎盤3.96 ± 0.39%；細胞凋亡與Ki-67的表現在子癲前症病人較正常者來的顯著明顯。而Bcl-2的IRS在子癲前症與正常者分別是3.70±0.86和4.03 ± 1.25，子癲前症雖較正常者低，但統計學上無明顯意義。

結論： 子癲前症胎盤的滋養層細胞Apoptosis及Ki-67的表現皆比正常者明顯增加，其原因可能和anti-apoptotic factor（Bcl-2 protein）表現減少，或是因為胎盤絨毛血管內膜細胞功能異常造成組織缺氧。可見這些因子的表現在子癲前症的病理機轉上扮演著一定角色。

（童綜合醫誌 2009; 3: 67-74）

關鍵詞：子癲前症、滋養層細胞、細胞凋亡、增殖、TUNEL

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前　言

在美國約有7%到10%懷孕的婦女有子癲前症（Pre-eclampsia）[1]，它是造成胎兒罹病及死亡最常見原因，及懷孕母親死亡的第二常見因素[2]；這是一種只發生在人類身上的病[3]，定義爲本來正常無高血壓的婦女，在懷孕20週以後，血管收縮壓超過140mmHg且舒張壓超過90mmHg，且需連續兩次間隔4小時以上皆不正常，同時24小時尿液中的蛋白超過300mg或尿液試紙蛋白尿超過1+[4]。子癲前症確實發生的病理機轉目前尚未完全明瞭，而終止妊娠將胎盤移除母體，母體血壓便急速恢復正常。可見胎盤是否正常和子癲前症的發生有著密切關係。

胎盤是一種有如腫瘤般（tumor-like）的器官，滋養層細胞會隨著懷孕時間的增加而增殖、移行、侵入至子宮，但卻又不像癌症般無限制成長；當懷孕9個月後胎盤逐漸發生鈣化現象，功能也開始減少，到妊娠42週時更是急速下降。爲何會有如此變化，我們相信和細胞凋亡有關[5]。1972年Kerr[6]等提出細胞凋亡的概念，細胞凋亡又稱謂細胞程序化死亡（programmed cell death），透過這種基因調控機制，身體完成衰老與畸形細胞的清除。胎盤細胞經過酵素活化（enzyme activation），或蛋白質表現（protein expression）啓動了計劃性的細則死亡。Stephen C Smith et al.[7]於1997年第一位研究胎盤在不同懷孕週期的變化，發現胎盤細胞凋亡發生比例在妊娠末三個月（third-trimester）比妊娠前三個月（first-trimester）來的明顯，胎盤這種一方面凋亡，一方面持續增殖維持其功能，推測凋亡及增殖的發生對於胎盤的成長發育及病變扮演著重要角色。

本文研究目的是針對子癲前症病人胎盤絨毛上滋養層細胞，研究其細胞增殖與凋亡兩者的表現與正常胎盤是否有所不同，藉此來了解及解釋子癲前症的可能病理機轉。

材料與方法

於2006年7月至2007年6月，在童醫院生產的14位孕婦的胎盤（7個爲正常者胎盤，7個爲經診斷爲子癲前症的胎盤），生產方式皆爲剖腹生產，採樣時間爲生產胎盤取出後。迅速選取胎盤母體面靠近臍帶處（避開鈣化或不正常出血處）約1.0cm × 1.0cm × 1.0cm小塊，放入10%中性福馬林固定。生產前已先向孕婦解說實驗用途，並取得胎盤採樣同意書。子癲前症定義爲本來正常無高血壓的婦女，在懷孕20週以後，血管收縮壓超過140mmHg且舒張壓超過90mmHg，且尿液試紙蛋白尿超過1+。對照組爲無高血壓、糖尿病或其他內科疾病史，尿液試紙皆無蛋白尿。

爲評估比較子癲前症與正常者胎盤滋養層細胞在凋亡差異，我們使用TUNEL stain（TdT-mediated dUTP-biotin nick end labeling，脫氧核糖核苷酸末端轉移媒酶介導的缺口末端標記法）及免疫組織化學染色法（Immunohistochemistry stain；IHC）分析Bcl-2。爲比較兩者在胎盤滋養層細胞增殖不同，利用的標誌爲Ki-67。

胎盤組織取得經過脫水、包埋、切片與組織染色（Hematoxylin & Eosin stain；H&E stain）免疫組織化學染色法（Immunohistochemistry stain）後於光學顯微鏡下（Olympus，BX50）觀察並照相。在光學顯微鏡20 × 10倍率下，避開明顯發炎或鈣化地方，本人隨機任取5個視野，計算經免疫組織化學染色法染上的滋養層細胞（例如染成棕色）數目，除以在計算該視野下正常滋養層細胞數目再乘以100（染色細胞/正常細胞X100）%，即代表百分率。Semiquantitative immunohistochemical Remmele score（IRS）[13]評估免疫化學染色強度（intensity）和分佈範圍(distribution patterns)，將光學顯微鏡下染色細胞強度分成四級：0爲無染色；1爲微弱（weak）；2爲中度（moderate）；3爲強度（strong staining）。細胞分佈定義爲染色細胞所佔百分比：0爲無染上；1爲少於10%細胞被染色；2是11-50%；3是51-80%；4是大於81%。將兩者分數相乘即爲IRS.統計分析軟體爲Statistical Package for Social Sciences（SPSS^{TM}）for Window XP version 11，本研究實驗數據以平均值±標準差（Mean ± SD）來表示。在比較各組間差異時。P值<0.05爲統計學上有顯著差異。（Mann Whitney U test）

結　果

1. 孕婦臨床資料

實驗共收集7位子癲前症病人胎盤及7位正常剖腹生產者胎盤，這14位孕婦的臨床資料如表一所示，分析其基本資料後發現：母親生產體重在子癲前症病人比正常者來的重（表二），懷孕妊娠週數及胎兒出生體重在子癲前症病人則明顯不足正常者，胎兒出生狀況（Apgar score）在子癲前症病人，分數也明顯較低。而子癲前症者胎盤重量比正常者平均較重，但這並無達到統計學意義。

2. Apoptosis Expression

細胞凋亡的評估是以TUNEL 方法來表現，在滋養

表1 14位孕婦的臨床資料
Clinical characteristics

Case	Clinical category	Parity	Gestation(wk)	Reason for delivery	fetus weight(gm), sex	Apgar score
1	Normal	1	39	previous cs	3080, F	9 -->10
2	Normal	1	38	previous cs	2968, F	8 --> 9
3	Normal	1	38	previous cs	2878, F	9 -->10
4	Normal	1	38	previous cs	3150, M	9 -->10
5	Normal	1	37	previous cs	3200, M	9 -->10
6	Normal	1	38	previous cs	2887, F	9 -->10
7	Normal	0	38	breech	3194, M	9 -->10
8	MP	0	38	elective cs	2964, M	7 --> 8
9	MP	0	37	elective cs	3182, M	8 -->9
10	SP	3	32	emergent cs	1600, F	7 --> 8
11	SP	0	34	emergent cs	1748, F	7 --> 8
12	SP	0	37	emergent cs	1860, M	7 --> 8
13	MP	0	35	elective cs	2450, M	8 --> 9
14	MP	0	38	elective cs	3100, F	9 -->10

MP: mild preeclampsia. SP: severe preeclampsia.
cs: cesarean section. M:male, F:female

表2 子癲前症病人和正常者孕婦臨床資料的比較
Comparison of clinical characteristics

Characteristics	Normal (n=7)	Preeclampsia(n=7)	p-value
Maternal age(years)	30.1 ± 4.3	31.7 ± 4.1	NS
Maternal height(cm)	156.7 ± 8.3	158.8 ± 5.4	NS
Maternal weight(kg)	68.9 ± 11.4	83.6 ±11.9	0.03
Parity	0.8 ± 0.3	0.4 ± 1.1	NS
Gestation age(weeks)	38.0 ± 0.6	35.8 ± 2.2	0.03
Fetus weight(gm)	3051.0 ± 139.6	2414 ± 680.3	0.02
Placenta weight(gm)	635.5 ± 115.9	554.0 ± 142.8	NS
Apgar-1	8.8 ± 0.3	7.5 ± 0.7	0.02
Apgar-5	9.8 ± 0.3	8.7 ± 0.7	0.04

Data are presented as mean ± SD
NS, not significant ($p > 0.05$)

表3 子癲前症滋養層細胞內細胞凋亡統計與正常比較
Apoptotic Nuclei in Placental trophoblast Samples

Nuclei	Preeclampsia (n=7)	Control (n=7)	P
Total apoptotic nuclei	9.0 ± 0.8	5.2 ± 1.4	<.01
Total nuclei	2495 ± 112.4	2631 ± 137.7	NS
Apoptotic index rate(%)	0.37 ± 0.04	0.18 ± 0.05	<.01

Data are presented as mean ± SD
NS, not significant (p > 0.05)

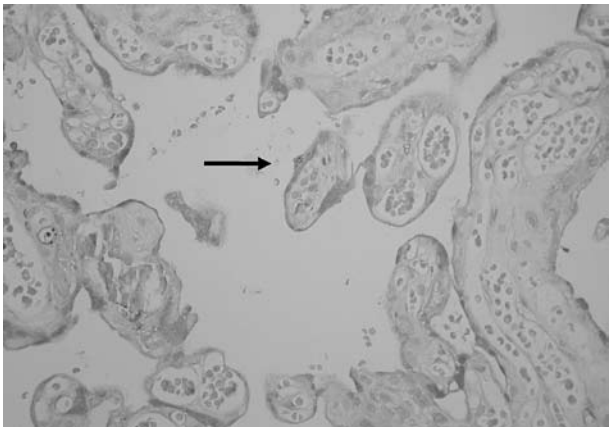

圖1 胎盤滋養層細胞經TUNEL 染色
背景爲藍色，陽性地方爲棕色（箭頭所指），原始放大倍數 X 200。

層細胞經TUNEL stain爲棕色者代表陽性（圖一），在子癲前症胎盤裡陽性比例平均值0.37% ± 0.04%，而正常者爲0.18% ± 0.05%（表三），具統計學意義。

3. Bcl-2 Expression

Bcl-2 protein 免疫組織化學染色在子癲前症與正常者胎盤trophoblast cells如（圖二），經IRS（Semiquantitative immunohistochemical Remmele score）分析兩者平均值（圖三），在子癲前症胎盤裡陽性比例平均值4.03±1.25，而正常者爲3.70±0.86，雖然子癲前症滋養層細胞質內Bcl-2蛋白表現較正常者較低，但並無統計明顯顯著。

4. Ki-67 Expression

任何深棕色細胞核染色代表是陽性反應（圖四），計算在陽性反應細則核數目所占所有細胞百分比後（staining index），子癲前症滋養層細胞Ki-67 positive index 是4.60±0.20%，正常胎盤則爲3.96±0.39%，子癲前症表現比正常者較明顯（表四），P<0.05。

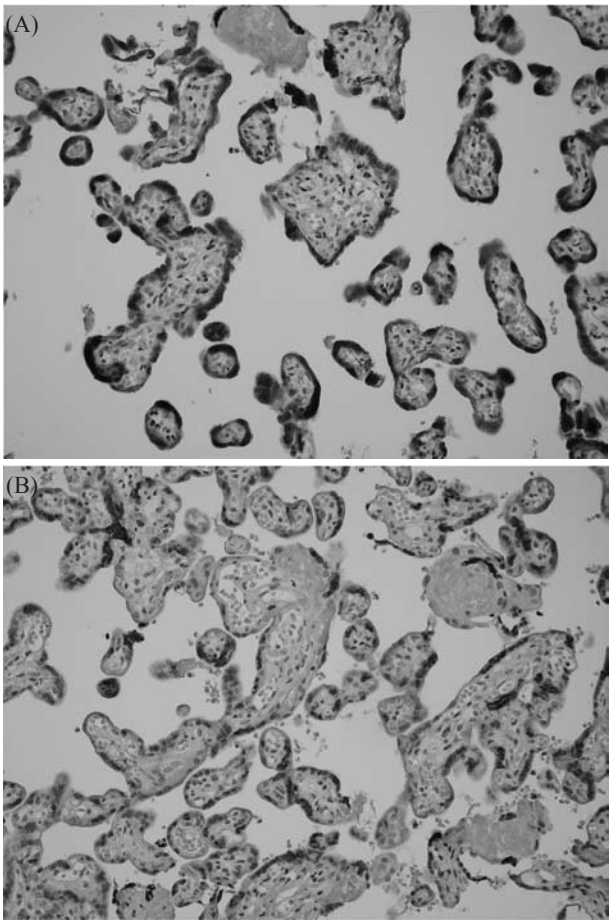

圖2 Bcl-2蛋白在胎盤滋養層細胞的細胞質經免疫組織化學染色
正常胎盤(A) 與子癲前症(B) BCL-2蛋白在細胞質（染成棕色）的表現（原使放大倍率 X 200）

表4 子癲前症滋養層細胞內細胞Ki-67的表現與正常者比較
Ki-67 Nuclei in Placental trophoblast Samples

Nuclei	Preeclampsia (n=7)	Control (n=7)	P
Total Ki-67 nuclei	113.8 ± 5.1	99.1 ± 8.9	0.03
Total nuclei	2471.4 ± 65.5	2501.2 ± 62.7	NS
Ki-67 stain index(%)	4.60 ± 0.20	3.96 ± 0.39	0.03

Data are presented as mean ± SD(standard deviation)
NS : not significant

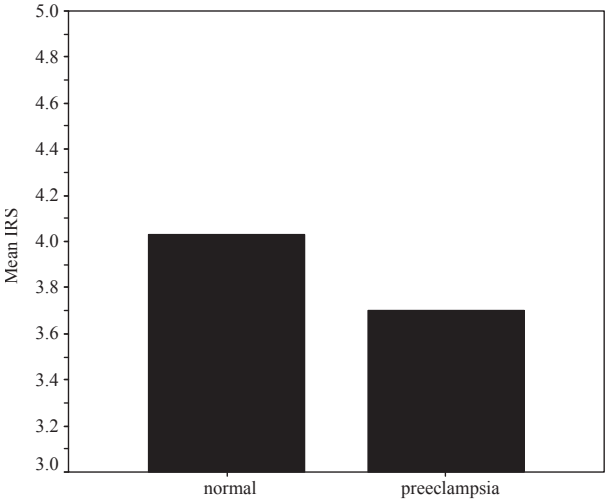

圖3 子癲前症胎盤滋養層細胞IRS與正常者的比較
在子癲前症胎盤滋養層細胞IRS陽性比例mean ± SD:4.03 ± 1.25，而正常者爲3.70 ± 0.86（Not significance）

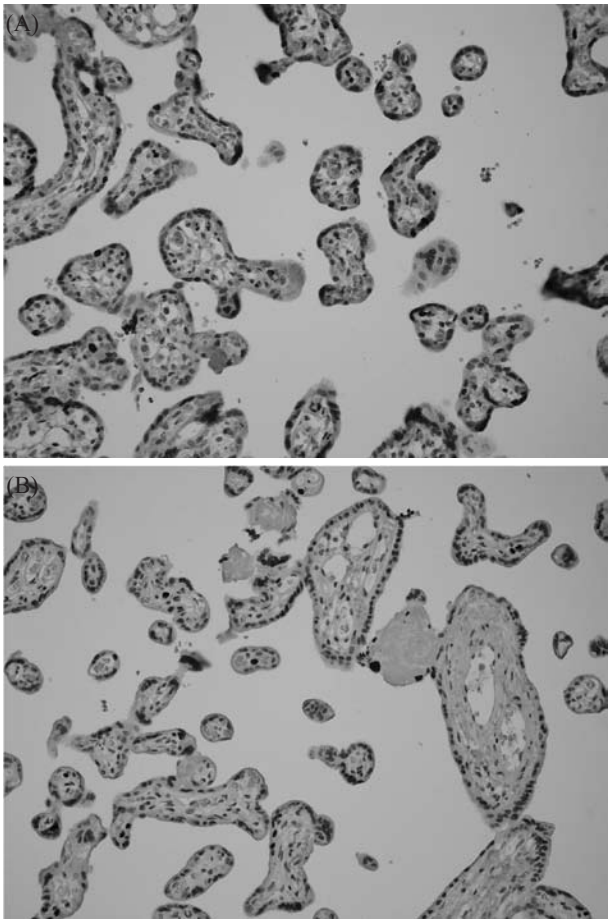

圖4 Ki-67在胎盤滋養層細胞內經免疫組織化學染色法
深棕色細胞核染色代表是陽性反應，上圖爲正常胎盤，下圖爲子癲前症。（原使放大倍率 X 200）

討論與結論

子癲前症是一種多發性器官的異常造成，從單純懷孕20週後血壓增高，腎臟腎絲球過濾異常尿蛋白質流失，到血液溶血（hemolysis），肝功能異常（elevated liver function），低血小板症狀（合稱HELLP syndrome），甚至癲癇發生。目前對於它的病理致病機轉仍未完全了解，但是胎盤內膜上皮細胞功能異常（endothelial cell dysfunction）[8]則絕對是一個關鍵因素。

胎盤的凋亡是一種正常的生理現象，相較於first-trimester的胎盤，third-trimester的胎盤發生凋亡機率的確是比較高，而發生位置也不因距離臍帶遠近而影響[9]。雖然診斷細胞凋亡可以藉由顯微鏡下的特殊形態（例如：condensation of nuclear heterochromatin, first as a crescent apposed to nuclear membrane, later into single or multiple dense bodies），但這並不是簡單的事，而電子顯微鏡（electron microscopy）則是鑑別凋亡的“gold standard”[10]，但昂貴器材並不是容易取得。本實驗標本先經過H&E（Hematoxylin – Eosin）stain，但在光學顯微鏡下並

無法確實區分兩者明顯異常凋亡。再採用TUNEL方法評估胎盤凋亡。正常胎盤的發生率，在使用TUNEL檢測的median rate 是0.51%[11]，我們結果是正常胎盤爲0.18%，子癲前症則爲0.37%，雖然較其他學者研究結果較低；但子癲前症較正常胎盤的確較明顯。目前已經證實，子癲前症的胎兒會有生長遲緩（intrauterine growth retardation）和子宮胎盤的循環不良，增加組織的缺氧及凋亡。

Bulmer[12]發現大部份的滋養層細胞核內有Ki-67的活性。Gerard[13]更是發現隨著懷孕週數的增加，Ki-67的表現逐漸減少。欲研究滋養層細胞生物活性，Ki-67被認爲是具有高準確度的標誌[14]，我們的研究結果發現，子癲前症的胎盤滋養層細胞Ki-67的表現比正常者明顯，這結果和其他學者相同[15]，原因爲何？目前尚未定論，認爲可能因爲子癲前症的胎盤滋養層細胞受到傷害，爲了修復受傷細胞，促使細胞核增殖反應，Ki-67活性也增加。

研究限制：在利用光學顯微鏡分析計算結果時，由於所計算數目不少，例如一個病人胎盤就數超過2000個細胞，長時間下來或許增加判斷上的誤差。本篇研究共取14位胎盤組織，來源數目仍稍不夠，另外如果能更進一步分析做蛋白質萃取分析，則像BMP（bone morphogenetic protein）family, CD34, VEGF（vascular endothelial growth factor），則會更是完美。

結論：比較子癲前症病人胎盤滋養層細胞與正常者表現發現：細胞凋亡發生率、及Ki-67的表現在子癲前症較正常高，並據統計學上的意義，而Bcl-2 protein表現則較低（雖然統計學上無明顯意義）。本研究顯示Apoptosis與Ki-67在子癲前症胎盤的機轉表現，扮演重要角色。

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Apoptosis and Proliferation index in the Placental Trophoblasts Between Normal and Preeclampsia Women Compared

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Background and purpose: Preeclampsia is characterized by hypertension, edema, and proteinuria and its etiology remains unknown. Trophoblastic cell play an important role in the maintenance of placental function. However, information regarding to the roles of apoptosis and proliferation in the trophoblast of placenta in relation to the pathophysiology of preeclampsia. The aim of the study was to compare the difference between preeclampsia has been scanty and normal term pregnancy placenta in the expression of some proliferation and apoptotic/anti-apoptotic markers in the human placenta.

Materials and Methods: Placental samples were obtained form 7 normal uncomplicated term pregnancy and 7 preeclampsia patients. Placenta samples were collected during cesarean section. Apoptosis was assessed by the terminal deoxynucleotidyl transferase deoxy-UTP-nick end labeling(TUNEL) method. Expression of Ki-67, Bcl-2, were assessed using immunohistochemistry. The positive index rate (%) was defined as (positive stain cells/ total nuclei) X 100 and expressed as mean ± SD. The Bcl-2 was assessd by semiquantitative Immunohistochemical Remmele Score(IRS). For each placenta, 5 randomized fields are examined by light microscopy at a magnification of x 200(x 20 objective lens and x 10 eyepiece). Statistical significance was determined by using analysis of variance to compare the rates between normal and preeclampsia placentas. Statistical analysis was performed with the use of Mann Whitney U test. A p value of <0.05 was judged as significant.

Result: The TUNEL-positive cells of the placenta were trophoblast with cluster of nuclei and the TUNEL-positive index of these cells was 0.37% ± 0.04% and normal placenta was 0.18% ± 0.05%. The Ki-67-positive index cells was 4.6% ± 0.2% and normal placenta was 3.9% ± 0.3%. The incidence of apoptosis and Ki-67 expression were significantly higher in preeclampsia placentas when compared with normal controls. Conversely Bcl-2 expression was lower generally in preeclampsia than nor-

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mal, albeit no significant difference between the two groups was observed.

Conclusion: These results suggest that trophoblast proliferation and apoptosis index may play a role in the pathophysiologic mechanisms of preeclampsia.

(Tungs' Med J 2009; 3: 67-74)

Key words: preeclampsia; trophoblast; apoptosis; proliferation; TUNEL

Comparative Assessment of the Diagnostic Accuracy of Serum Cystatin C, β-trace Protein and Creatinine for Predicting the Early Renal Function Impairment in Elderly Critically Ill Patient

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Background: In critical ill patients, acute kidney injury is often associated with a high mortality rate. A plethora of studies described about early marker of acute kidney injury and serum cystatin C, β-trace-protein had been showed to be more accurate than creatinine. However, there is no confirmatory study being conducted in Taiwan. Our study is therefore to compare the performance of serum creatinine, cystatin C (Cys C), and β-trace-protein (BTP) concentration for predicting early renal impairment in elderly patients.

Methods: This prospective study enrolled 102 patients in intensive care unit. Twenty-four hours creatinine clearance (CCr) and the serum creatinine, Cys C, and BTP were analyzed. All data were adjusted and compared with CCr.

Results: The mean age of this group of patients was 77.3 ± 12.7 years old. The inverse of serum Cys C ($r = 0.654$, $p < 0.001$) correlated better with CCr than did 1/BTP ($r = 0.384$, $p < 0.001$) as well as 1/creatinine ($r = 0.040$, $p = 0.629$). Most importantly, we found that serum Cys C is also a superior biomarker than creatinine or BTP for early detection of renal impairment.

Conclusion: In the critically ill elderly patient, serum Cys C appears to be a better marker for prediction of early renal function impairment.
(Tungs' Med J 2009; 3: 75 - 83)

Key words: Cystatin C, β-trace protein, renal impairment, elderly, critical

INTRODUCTION

Acute kidney injury (AKI) is common in critically ill patients and is often associated with a high mortality rate. Early detection of acute kidney injury is critical to alleviate its progression[1] and to allow for earlier intervention. In routine practice, the glomerular filtration rate (GFR) is used to evaluate the renal function. The most ideal marker to estimate the GFR was endogenous inulin clearance because inulin had stable productive rate, lack of reabsorption and excretion, and free filtration to glomerulus. However, it

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was not suitable and practical for routine use in critically ill patients because of its invasiveness. There were other biochemical parameters to estimate GFR. Among these parameters, the serum creatinine is the most commonly used. However, there are limitations for using creatinine. Serum creatinine is imprecise to detect the early-impaired GFR because the serum creatinine is usually less than 1.3 mg/dl in patients with GFR less than 60ml/min/1.73m^2, especially in female[2]. Furthermore, serum creatinine could be influenced by factors such as age, gender, weight, etc. In intensive care unit, twenty-four hours creatinine clearance (CCr) is often used to estimate the renal function in critically ill patients. Again, there are some limitations to its use, such as adequacy of urine collection, a time-consuming method and not a real-time monitor of GFR. The risk of mortality associated with advance acute renal injury is distressingly high. Studies of animal models of ischemic and septic AKI have suggested that beneficial effects of therapeutic agents that attenuate renal injury were effective only if the agents were administered before or early in the course of AKI[3, 4]. Hence, if patients at high risk of developing AKI can be diagnosed early, then relevant and preventive therapy can be initiated expediately.

Owing to the importance of earlier therapeutic intervention, some markers have been evaluated for the early detection of renal dysfunction in critically ill patients. Cystatin C (Cys C), an endogenous cationic low-molecular weight protein (pI: 9.3, molecular mass: 13.3 kDa) is almost freely filtered across the glomerular membrane where it is reabsorbed and catabolized almost completely in proximal renal tubular cells and only 0.5% appears in the urine[5-7]. Cys C is produced by all nucleated cells at a constant rate [8], with age, sex and body composition having very little effect on its serum concentration[9]. Cys C is not affected by infections, liver diseases, or inflammatory diseases[10]. Several studies had documented that cys C is a better predictor than creatinine for prediction of early GFR impairment[1, 5]. One study report that a comparison between Cys C and creatinine for estimation of GFR (determined with [^{125}I] iothalamate) showed the correlation between inverse of Cys C and GFR is highly significant [r =0.873, 95% confidence interval (CI) 0.828 –0.907, p < 0.0001] [5].

Since 1985, Cys C has been suggested to be a marker of renal function. Nowadays, several studies reported that serum Cys C was a better marker than creatinine for early detection of acute renal failure[1, 5, 11-18].More recently, another low molecular weight protein, β-trace protein (BTP), was isolated primarily from cerebrospinal fluid (CSF), known as prostaglandin D synthase, and was a 23- to 29 kDa enzyme and belongs to the lipocalin protein family. BTP was shown to be increased in patients with renal impairment and proved to be a useful and an accurate marker to detect the CSF rhinorrhea and otorhea (CSF leakage)[19, 20]. Recently, some studies have shown that serum BTP seem to be a better marker than creatinine for the assessment of reduced glomerular filtration rate (GFR) in renal transplant patients[12, 19-24]. In addition, BTP has also been proposed as an alternative marker for GFR in renal transplantation patients, in children and in persons with diabetes or various renal diseases[12, 21-24]. Furthermore, one study showed that BTP is superior than serum creatinine and comparable for Cys C to detect mildly reduced GFR in children[12].

Thus, the objective of our study is to evaluate the diagnostic accuracy of Cys C, BTP, creatinine and multivariate serum creatinine/Cys C-based formulae for creatinine clearance in critically ill patients.

MATERIALS AND METHODS

The patient group consists of those who admitted to the medical intensive care unit and coronary care unit at the Tungs' Taichung MetroHarbor Hospital between March and July 2007. The inclusion critera used in our study was based on the serum creatinine, less than 1.2mg/dl at admission. All patients had been given their informed consent and the institute committee on human research had approved the study protocol.

Patients with serum concentration of creatinine above 1.2 mg/dl within the past 6 months or patients who expired within 24 hours after enrolling were excluded. All patients who were enrolled had baseline demographic, clinical and biochemical data obtained. A 24-hour urine sample was obtained when the patient admitted to intensive care unit. A serum sample to measure the serum creatinine (Scr), Cys C (Scy), and BTP (Sbtp) was drawn and stored at the time of completing the 24 hours urine collection. Body surface area [BSA (Mosteller formula)] was defined as body weight (kg) x body height (cm))$^{1/2}$ to ml/min per

1.73 m^2. Serum creatinine was measured by a modified Jaffé method (Hitachi 7170) while serum Cys C level were measured by immunonephelometric method (Company, State Country) on the Hitachi 7170 analyzer. BTP was measured with a latex-particle–enhanced immunonephelometry assay (Dade Behring) N Latex BTP nephelometric test. All samples were rechecked for creatinine, Cys C and BTP. The final value was decided by mean value of the two values in identical parameter.

We chose some common clinical formulae based on creatinine and Cys C to estimate the GFR as following:

Estimation of renal function (all expressed as ml/min per 1.73m^2 except Modification of Diet in Renal Disease (MDRD) - GFR)

24 hours creatinine clearance (CCr) = (urine amount x urine creatinine) / (serum creatinine x 1440) x (1.73/BSA).

Creatinine-based formulae

The estimated GFR using serum creatinine (Scr) is calculated by below formulae

(1) CG-GFR (Cockcroft-Gault) = ((140-Age) × Body weight (kg))/ (72 x serum creatinine) x (1.73/BSA) x 0.85 (if female).

(2) MDRD (Modification of Diet in Renal Disease) -GFR = $186 \times Scr^{-1.154} \times Age^{-0.203} \times 0.742$ (if female)

Cystatin C–based formulae

The estimated GFR using serum Cys C (Scy) is calculated by below formulae

(3) Le Bricon-GFR[26] = 78÷Scy + 4.

(4) Hoek-GFR[11, 26] = – 4.32+80.35÷Scy.

(5) Larsson-GFR[27] = $77.24 \times Scy^{-1.2625}$ x 1.73/BSA.

Normal reference of serum creatinine range from 0.6 to 1.3mg/dl, serum Cys C range from 0.6 to 1.0 mg/l and BTP from 0.4 to 0.9 mg/l in our hospital.

Statistical analysis was performed using SPSS 14.0 for windows. The data are expressed as a mean value ± 1 standard deviation. The correlations between the data were using the Pearson's correlation test. The Cys C-based or creatinine-based formulae were correlated with CCr. $P < 0.05$ was considered statistically significant. The diagnostic value of Cys C, creatinine, BTP to identify early-impaired renal function were obtained using the receiver operating characteristic (ROC) curve analysis and area under curves (AUCs, expressed as Mean ± 1 SD).

RESULTS

A total of 102 patients were included in our study, the mean age was 73.33 ± 12.7 years old and 39% of the cohort was female. The demographics, biochemistry laboratory result and clinical characteristics of the patients were summarized in Table 1 and 2. The mean serum concentration of creatinine, Cys C and BTP were 1.02 ± 0.38 mg/dl, 1.34 ± 0.51mg/l, respectively and 0.67 ± 0.39 mg/l in our cohort. The mean CCr was 67 ± 47.71 ml/min/1.73m^2.

The correlation between each parameter and CCr were summarized as Table 3. The serum Cys C and BTP level were significantly correlated with serum

Table 1. Demographic characteristics and laboratory data of the studied patients

Parameter	Value (mean ± SD)
Age	73.33±12.70 (42-101)
Sex (M/F)	62/40
ACACHII score	17.75±5.65 (5-31)
Creatinine (mg/dl)	1.02 ± 0.38 (0.45–3.80)
Cystatin C(mg/l)	1.34 ± 0.51 (0.42–3.46)
β-trace protein (mg/l)	0.67 ± 0.39 (0.22–2.74)
Formulae	
Creatinine-based	
24 Hr CCr, adjusted by BSA	67.09 ± 47.71 (6.53–239.03)
CG-GFR, adjusted by BSA	58.25 ± 21.77 (10.52–140.24)
MDRD-GFR	77.19 ± 28.12 (11.94–200.93)
Cystatin C-based	
Le Bricon-GFR	69.35 ± 22.24 (26.58–191.95)
Hoek-GFR	63.00 ± 22.91 (18.94–189.29)
Larsson-GFR	62.90 ± 27.78 (16.15–234.45)

Abbreviation: CCr: 24 hours urine creatinine clearance, GFR: glomerular filtration rate, M: male, F: female, CG-GFR: Cockcroft-Gault-GFR, BSA: body surface area, MDRD: Modification of Diet in Renal Disease-GFR

Table 2. Demographic characteristics and laboratory data related to CCr

CCr	No of subjects	Creatinine(mg/dl)	Cystatin C(mg/l)	Β trace protein(mg/l)
>90	19	0.98±0.13 (0.80-1.30)	0.92±0.18 (0.42-1.25)	0.47±0.12 (0.30-0.74)
90-70	14	0.94±0.25 (0.60-1.35)	1.06±0.17 (0.87-1.41)	0.47±0.19 (0.22-0.79)
70-50	27	0.91±0.24 (0.45-1.30)	1.22±0.23 (0.81-1.79)	0.56±0.16 (0.29-0.91)
50-30	29	0.97±0.22 (0.60-1.50)	1.50±0.36 (0.93-2.29)	0.73±0.29 (0.34-1.69)
<30	13	1.45±0.82 (0.50-3.80)	2.15±0.68 (1.07-3.46)	1.25±0.68 (0.29-2.74)

Abbreviation: CCr: 24 hours urine creatinine clearance

Table 3. Correlation between CCr and each separate parameter

parameter	Pearson's correlation	P value
1/Scr	0.040	0.629
1/Scy	0.654	<0.001
1/Sbtp	0.384	<0.001
MDRD-GFR	0.221	0.025
CG-GFR	0.441	<0.001
Le Bricon-GFR	0.654	<0.001
Hoek-GFR	0.654	<0.001
Larsson-GFR	0.645	<0.001

Abbreviation: CCr: 24 hours urine creatinine clearance, Scr: serum creatinine, Scy: serum Cystatin C, Sbtp: serum β-trace protein level, GFR: glomerular filtration rate

creatinine (0.561 and 0.594, $p < 0.001$, respectively). Besides, the BTP level also significant correlated with Cys C (0.693, $p < 0.001$). The adjusted CCr is significant correlated to 1/Scy, 1/Sbtp, adjusted CG-GFR, Le Bricon-GFR, Hoek-GFR, Larsson-GFR (all $p < 0.01$) and MDRD-GFR ($p < 0.05$). In our results, correlation between CCr and 1/Scy ($r = 0.654$, $p < 0.001$) or 1/Sbtp ($r = 0.384$, $p < 0.001$) is significant. Serum creatinine level seems to be not correlated to CCr ($r = 0.040$, $p = 0.629$).

Among the creatinine-based and Cys C-based formulae (against CCr), Le Bricon-GFR ($r = 0.654$, $p < 0.001$), and Hoek-GFR ($r = 0.654$, $p < 0.001$) had the best correlation.

The ROC curve analyses and area under curves of serum creatinine, Cys C and BTP were shown in Fig 1a-d. Cys C is a better predictor than BTP and creatinine in all subgroup. Serum creatinine is a very poor predictor even in CCr < 50 ml/min/1.73m^2.

According to CCr, patients were also stratified into 5 groups: CCr＜30, 30≦CCr＜50, 50≦CCr＜70, 70≦CCr＜90, and ≧90ml/min/1.73m^2 (simplify the label as CCr＜30, 30-50, 50-70, 70-90, ＞90). The mean serum concentrations of creatinine, Cys C, and BTP in each group were listed in Table 2 and in Fig. 2. Serum Cys C showed a significant increase in the group: "70-90" (compare to ">90" group, $p = 0.034$) and BTP showed a significant increase in the group "30-50" (compare to "50-70" group, $p = 0.013$). Serum creatinine elevated significantly in group "<30" than group "30-50" ($p = 0.005$).

DISCUSSION

In critically ill patients, the real-time monitor of renal function was ulmost important to manage the underlying disease and hemodynamic status. One of the most accurate estimation is using injections of inulin or chromium EDTA and *p*-aminohippuric acid for the determination of the GFR and renal plasma flow. However, the disadvantages of these methods are invasive, cumbersome and involving the application of radioactive substance. Although 24 hours creatinine clearance may not give an exact measure of GFR, it is still a valuable gauge in serial assessment.

In our study, the inverse of serum creatinine concentration was a very poor marker for estimating CCr (r= 0.040, p = 0.629) and its level had significantly elevated only when CCr < 30ml/min/1.73m^2. Conversely, we found that serum Cys C ($r = 0.654$, $p < 0.001$), and BTP ($r = 0.384$, $p < 0.001$) were bet-

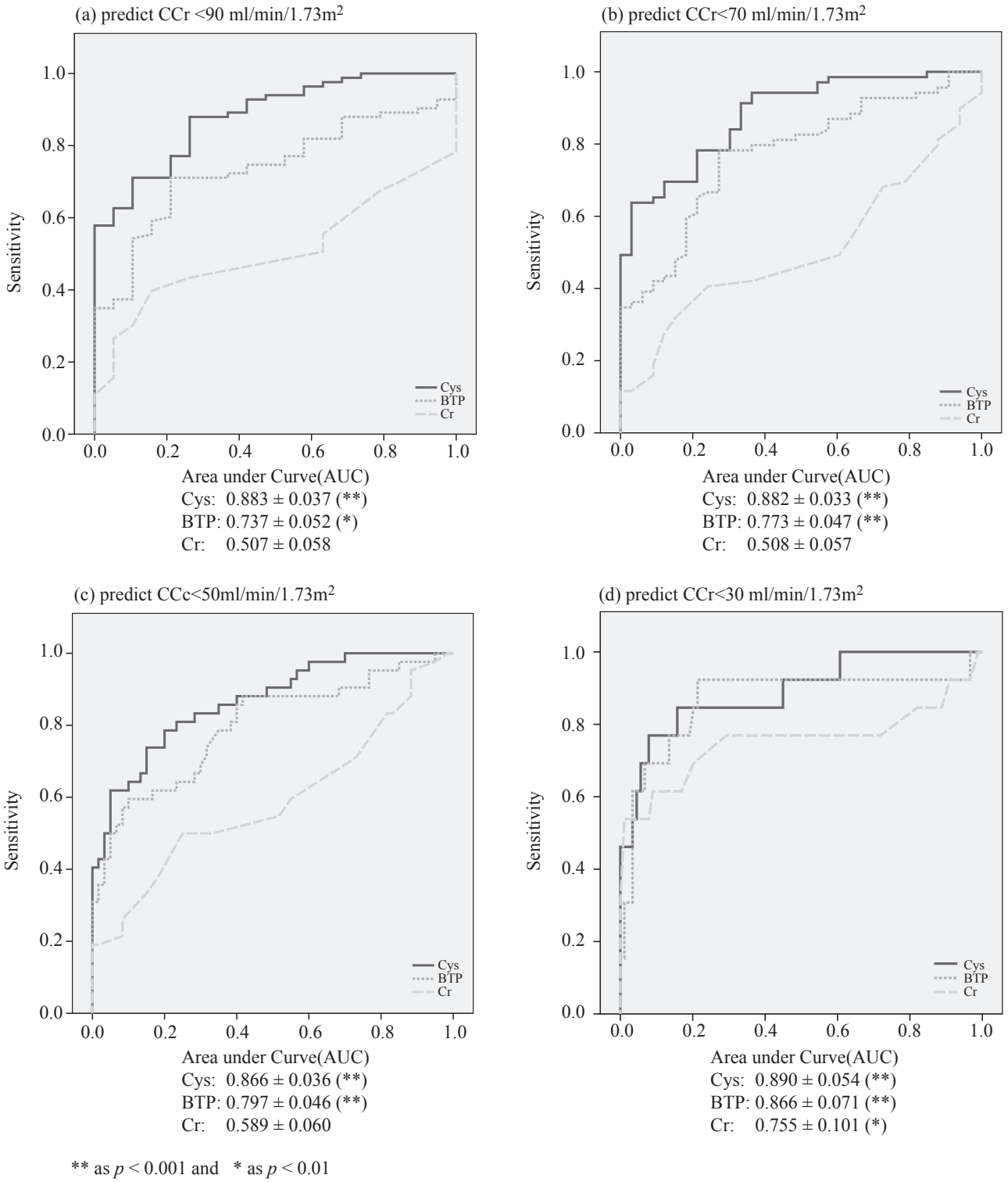

Fig. 1 The ROC curves and AUC of serum creatinine (Cr), cystatin C (Cys) and BTP (BTP) in different stage of renal function (ml/min/1.73m^2). AUC were expressed as area ± 1 standard deviation.

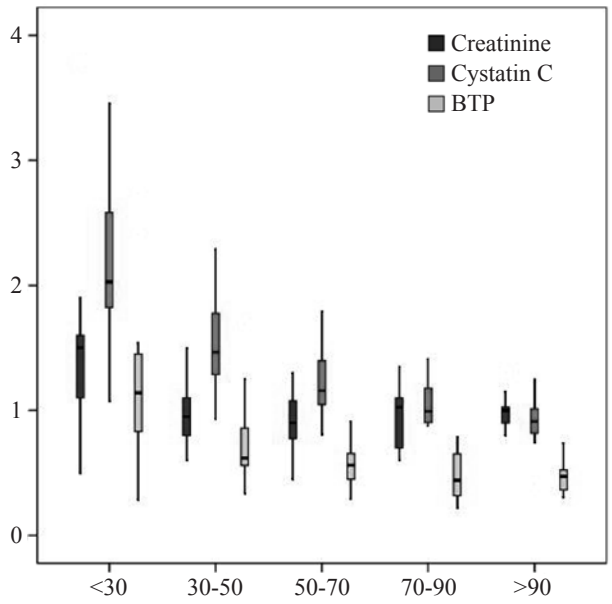

Fig. 2 The serum creatinine, cystatin C and BTP concentration at different stage of renal function. The renal function is divided into 5 groups as CCr < 30 (n=13), 30-50 (n=29), 50-70 (n=27), 70-90 (n=14), >90 (n=19) ml/min/1.73m^2. Data are given as box spots, where limits of the boxes indicate the 25th and 75th percentile and the lines inside the boxes indicate the 50th (median) percentile. The whiskers indicate the 90th and 10th percentile. The concentration of cystatin C started to elevate significantly at CCr: 70-90 than CCr >90 ml/min/1.73m^2 (p = 0.034) whereas BTP and creatinine aren' t elevated significantly (p = 0.867, p = 0.961). The BTP level started to elevate significantly at CCr: 30-50 compared to 50-70 ml/min/1.73m^2 (p = 0.013) whereas creatinine is not elevated significantly (p = 0.353). The creatinine level started to elevate significantly at CCr <30 than 30-50 ml/min/1.73m^2 (p = 0.005).

ter and more sensitive for estimating renal function. Comparatively, serum Cys C was the most sensitive method for detecting CCr in these critically ill patient as indicated by area under the curve of the ROC analysis and its level elevated significantly in very early stage of renal injury (when CCr < 90ml/min/1.73 m^2). Hence, elevated Cys C could early identify a subclinical state of change in renal function, which was not being able to detected by conventional methods. Conversely, serum BTP level showed significant elevation only when CCr < 50 ml/min/1.73 m^2.

In critically ill patients, particularly in the elderly, rapid changes in renal function usually occur and thus the rise of serum creatinine may lag behind the decline renal function and make it an unsuitable real time marker for early diagnosis of AKI. In one previous study, the author found that the 1/Scr had moderate correlation to CCr (r = 0.426, p = 0.002) in a group of ICU patients[5]. However, we did not observe such a correlation existed in our patients. One possible explanation for such discrepancy was that our patient was older than that group (Mean age was 73.3 years vs 54 years) and thus may had less muscle mass.

In our study, we had also attempted to correlate the creatinine-based formula with CCr. Cockcroft-Gault formula as well as MDRD formula were correlated with a certain extent to CCr with r = 0.441 (p<0.001), and 0.221 (p=0.025). We also found that the Cockcroft-Gault formula appeared to be better than MDRD formula in estimating the renal function of these critically ill patients. Clearly, both derived formula suffered from the shortcoming of Scr.

In 3 Cys C based formulae, both Le Bricon and Hoek formulae have the same correlation to adjusted CCr (r = 0.654, p < 0.001) which are slightly better than Larsson formula (r=0.645, p < 0.001). The Le Bricon and Hoek formulae are also simple and easy to use in clinical practice to predict the creatinine clearance.

In one reported study, estimated GFR by continuous [^{125}I] iothalamate method, the author found that the correlation between 1/Scy and GFR was highly significant [r=0.873; 95% confidence interval (CI) 0.828–0.907; p<0.0001] and significantly better (p =0.038) than between 1/Scr and GFR (r=0.800; 95% CI 0.733–0.852; p<0.0001). The high correlation of serum Cys C level with GFR permitted the calculation of a reliable formula for estimation of GFR from Cys C data[11].

BTP was documented to be independent of age and gender in one reported study[12]. There are some BTP-base formulae published to estimate the GFR in transplant patients. However, it is not sensitive or more accurate than Cys C in normal population in most studies[12, 20, 21,23-25]. However, in our study, BTP was better than creatinine but inferior to Cys C for predicting of renal status estimated by adjusted CCr.

Recent study showed that high Cys C concentra-

tions predict substantial increased risks of all-cause mortality, cardiovascular events, and incident heart failure among ambulatory persons with coronary heart disease[28].

CONCLUSION

Taken together, our study showed that serum Cys C appeared to be a better marker than serum creatinine and BTP for estimation of early impairment of renal function. In creatinine-based formulae, CG-GFR is better than MDRD-GFR to predict the creatinine clearance. Cys C-based formulae such as Le Bricon and Hoek formula are simple and accuracy for estimating creatinine clearance than other formulae. Hence, serum Cys C is a useful marker for early detection of change in renal function, especially in critical ill elderly patients. It also allowed for real-time monitor of alteration of renal function in these unstable patients.

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比較 Cystatin C, β-trace protein 及 Creatinine 在老年重症病人之早期腎功能不全的關聯性

吳再坤[1]　謝美智[2]　陳順良[3]　郭元銓[1]　劉家珊[1]　林柏松[1]*

背景：在急重症病人，急性腎臟損傷常合併死亡率的增加。本研究是比較cystatin C, β-trace protein 以及creatinine，三者之中何者可以較早期的預測腎臟功能不全。

方法：本研究共收錄了102位加護病房的病患，每位病患收集24小時creatinine清除率，並在尿液收集結束時抽取血液內之creatinine, cystatin C, β-trace protein 及creatinine值，並比較其相關性。

結果：病患年紀平均為77.3±12.7歲。與24小時creatinine清除率的關係性相比較，cystatin C的倒數（$r = 0.654$, $p < 0.001$）比β-trace protein的倒數（$r = 0.384$, $p < 0.001$）或creatinine的倒數有更好的相關性。此外，在偵測早期的腎臟功能不全，cystatin C比另外兩組有更加的準確度。

結論：在急重症老人的早期腎臟功能異常，cystatin C可能是個較佳的指標。
（童綜合醫誌 2009; 3: 75 - 83）

關鍵詞：cystatin C；β-trace protein；腎功能不全；老年人；急重症

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The Effects of Functional Electrical Stimulation (FES) Cycling Training on Muscle Strength and Standing Balance of Stroke Patients

Yung-Chun Hsu[1,4], Chun-Yu Yeh[2], Kuen-Horng Tsai[3]

Objective: To investigate the possible beneficial effects of muscle strength and standing balance in subjects with stroke using the FES-cycling ergometer system.

Methods: Sixteen hemiplegic stroke patients were randomly assigned to perform leg cycling exercise (LCE), one with functional electrical stimulation (FES) evoking muscle contractions during a single bout cycling training and a control group received the same study protocol without FES. All subjects, blinded for group assignment, were measured at baseline and at post-test in the laboratory. Muscle strength was quantitatively measured by hand-held dynamometer (HHD), and furthermore the standing balance was evaluated by the limits of stability (LOS).

Results: There was no significant change in the muscle strength after cycling in both groups. In addition, significant improvement of the paretic-side stability was detected in endpoint excursion (P= .031), maximum excursion (P= .000), and directional control (P= .013). While training-induced temporal changes of all outcome measures were no significant difference between the two groups.

Conclusions: This experiment showed that a short cycling training program is a useful therapeutic intervention to improve the standing balance ability in patients with stroke. But the use of FES had no additional benefit in this experimental study. In the future studies, it will be of interest to use larger groups of subjects to investigate the long-term effects of this intervention on motor recovery and functional walking.

(Tungs' Med J 2009; 3: 84-90)

Key words: functional electrical stimulation, leg cycling exercise, muscle strength, standing balance, stroke

INTRODUCTION

For post-stroke patients, varying deficits in sensation, strength, and balance in the paretic limbs sometimes disturb the activities of daily living. Although, recent studies have demonstrated that exercise can improve mobility[1-3] and functional balance[4,5] in patients with stroke, but it is unclear what the advan-

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tages of different types of exercise programs are and the mechanisms that underlie their improvements.

To enhance remaining functions of patients with partial motor disorders, functional electrical stimulation (FES) combined with cycling exercise has been exploited as a rehabilitation technology recently. Based on previous FES-cycling study results,[6-9] there have been demonstrated beneficial effects through increases in muscle mass and strength, circulation, and improved metabolic activity for spinal cord injured (SCI) patients.

Reduced lower limb function is common after stroke and is also an adverse prognostic behavior for personal welfare. However, few studies have attempted to investigate muscle strength and standing balance effects of FES-cycling exercise intervention in this population. The specific aims of this study were: (1) to attest whether a single bout cycling training for stroke patient can improve muscle strength and functional standing balance of paretic leg; (2) to demonstrate whether FES to the paretic leg during cycling exert more beneficial effects than cycling without FES.

METHODS

Subjects

Sixteen stroke patients (average age 48.4±10.6 years, range= 29-70 years, 13 male, 3 female) were recruited for the experiments. Inclusion criteria were as follows: (1) first-ever stroke with unilateral hemiplegia (Brunnstrom stage ≥3); (2) ability to stand without external support for at least 30 secs; (3) ability to understand and follow verbal commands; (4) no severe perceptual, cognitive, or sensory deficits; (5) no history of osteoarthritis, severe cardiopulmonary disease, or vascular disease in the lower limbs; and (6) without a fixed contracture in the paretic lower limb. Their average duration after the onset of stroke was 26.4±36.1 months. Seven suffered from cerebral infarction and nine from cerebral hemorrhage as judged by either computed tomographic scanning or magnetic resonance imaging, and eight patients had left and eight patients had right hemispheric lesions. Clinical details of the sixteen patients are shown in Table 1. All subjects gave their written informed consent as approved by the Ethics Committee of the Chung Shan Medical University School of Medicine after being fully informed of the details of the experimental procedures.

Training protocol

Before baseline testing, the subjects were randomly assigned to either the FES-LCE group or the LCE group without electrical stimulation. The initial assessment consisted of demographic details, stroke subtype, and the following measurements: muscle strength test (HHD); and standing balance test (LOS). After the 20-min training program, the same measurements were performed immediately.

Figure 1 shows the FES-cycling ergometer system that allowed the legs to be loaded in a stable balanced set. The system consists of four parts: the cycling ergometer, the ankle-foot orthosis (AFO), the angle encoder, and PC-based analysis system. In the cycling

Table 1. Demographic Data of Subjects in Both FES-LCE and LCE Groups

Characteristics	FES-LCE (n=8)	LCE (n=8)	*P*
Age (y)	46.1±9.9	50.8±11.4	.623
Gender (male/female)	6/2	7/1	1.000
Height (cm)	164.8±6.8	167.8±8.8	.698
Type of CVA (hemorrghage/ischemia)	5/3	4/4	1.000
Side of hemiplegia (left/right)	4/4	4/4	1.000
Time since CVA (mo)	27.0±43.1	25.7±30.6	.700

Note. Values are mean ± SD
Abbreviation: CVA, cerebral vascular accident.

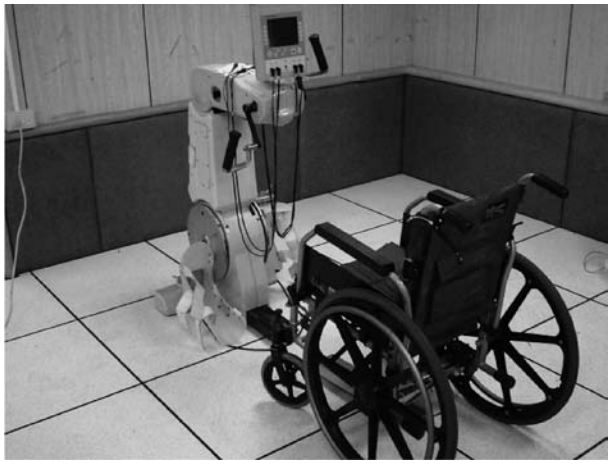

Fig. 1 The FES-cycling ergometer system.

ergometer, several parameters can be set, including the target cadence (45 rpm) and pedal resistance (level 10). Moreover, the ergometer raises the current amplitude to a predefined maximum when cadence falls below the target cadence. The purpose of this procedure is to recruit additional muscle fibers to maintain the target cadence.

Subjects in the FES-LCE group received a 20-min program of electrical stimulation to the paretic leg via surface electrodes over quadriceps and hamstring. Through the angular encoder, the current position of paretic leg could be detected. The FES controller stimulates the quadriceps and hamstring when the paretic leg sweeps past through the range from 26° to 170° and 200° to 290°, respectively.[10] The basic stimulation frequency and pulse width were set at 20 Hz and 300 μm.[11] The level of stimulation was increased until a comfortable gross muscle contraction was visible. Subjects in the LCE group were given the same training protocol except the FES. The training protocol and placement of electrodes were undertaken by a senior therapist.

Muscle strength

In analogy to many previous studies, hand-held dynamometer (HOGGAN Health Industries, Inc) has been reported as a reliable,[12,13] valid,[14-17] and sensitive[17,18] measure to quantify strength impairments in the upper and lower extremities post stroke. Maximal knee extension force of both legs was assessed while subjects were seated with hip angle at 90° and knee placed in 70° knee flexion (0° is full extension). The placement of the force plate, measured and multiplied the distance (from the tibial tuberosity to the superior aspect of the medial malleolus) by 0.6 was kept a tab on the anterior tibia.[19] Subsequently, subjects performed isometric maximal voluntary contractions (MVCs). Each contraction was held for 4 seconds. Three measurements were taken per rater with a 30-second rest between each measure. After a 3-minute rest period, the other rater repeated the procedure. For each rater, a mean value was calculated by averaging the 3 values.

Limits of stability

The Smart Balance Master system (Neurocom System Version 8.2.0) was used to measure the subjects' limits of stability and shown in Fig 2. This apparatus is a verified assessment tool to measure the path of the subject's center of gravity (COG) during exercise for analysis, which initiates and quantifies volitional movements.[20] The evaluated parameters included reaction time (RT), movement velocity (MVL), directional control (DCL), endpoint excursion (EPE), and maximum excursion (MXE). These evaluated parameters were assessed while subjects

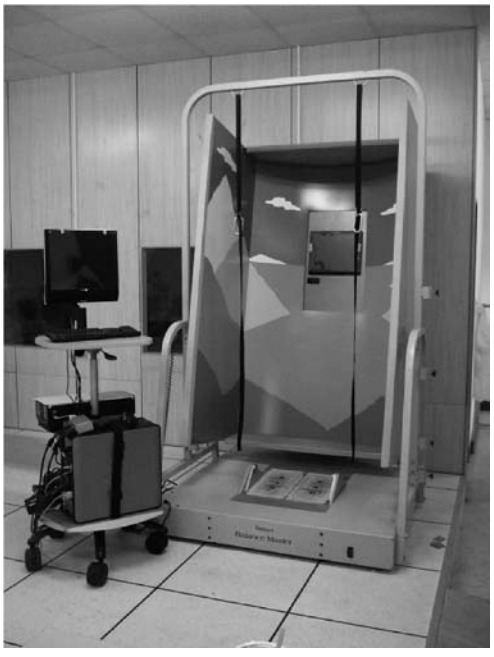

Fig. 2 The Smart Balance Master system.

can sway their body toward the paretic side target. RT is defined as the time in seconds between the signal to move and the subject's first movement; MVL is defined as the average speed of COG movement in degrees per second; DCL is defined as a comparison of the amount of movement in the intended direction (toward the target) to the amount of extrinsic movement (away from the target); EPE is defined as the distance of the first excursion toward the intended target (expressed in percent of maximum LOS); and MXE is defined as the maximum distance attained during the trial.[20] All subjects were instructed to stand on a standardized foot position of the force plate with their arms held in a relaxed position on the sides. Additionally, subjects were requested to move the COG cursor shown on the computer screen as quickly and accurately as possible for 8 seconds. Each participant had 5 minutes of familiarity with the system before executing the test.

Statistical analysis

The data were expressed in mean ± standard deviations (SD) and analyzed with SPSS (version 12.0). An independent sample *t*-test was used to compare the demographic data of both groups. Outcome measurements were analyzed with repeated measures ANCOVA to compare the main effects between groups and assessment intervals. Group assignment (FES-LCE, LCE) was the between-grouping factor, and assessment time (baseline, post-test) was the within-group factor, finally, the covariate was the time since stroke. Comparisons were considered significant when a *P* value less than .05 (2-tailed).

RESULTS

Demographics

Of the 21 subjects who met the inclusion criteria, 16 completed the study. Withdrawal before completion of the single 20-min training program occurred in 3 subjects in the FES-LCE group and 2 in the LCE group. There were no serious complications observed in subjects who had received the intervention treatment. Table 1 describes subject characteristics. Additionally, there were no differences between the two groups for baseline demographic data ($P > .50$).

Immediate training effects

Hemiplegic patient's responses to the training program in the main effects on muscle strength, and standing balance ability were shown and summarized in table 2. MVC force of the paretic knee extensors remained unchanged after intervention ($P = .456$), but MVC force tended to be somewhat declined in the sound knee extensors ($P = .073$). On the limits of stability, significant intervention effects were found in endpoint excursion ($P = .031$), maximum excursion ($P = .000$), and directional control ($P = .013$). Other parameters, including reaction time ($P = .321$) and movement velocity ($P = .291$), however, were not significant. Comparing the all outcome measures between the FES-LCE and LCE groups by using repeated measures ANCOVA analysis (Time × Group Interaction), no significant changes were presented (table 2).

DISCUSSION

The essence of our finding of this study was a significant improvement of standing balance, but not voluntary knee extensors muscle strength. Furthermore, FES of the paretic leg during cycling had no notable beneficial effects. Our results can be categorically elaborated as follows:

Muscle strength

By contrast, MVC force of the both knee extensors revealed no significant time effect or time-by-group interaction after training intervention. This result was consistent with a previous study,[21] which reported an exposition of the difference in the nature of the exercise (i.e., dynamic cycling involving relatively low forces of several muscle groups vs isometric knee extension involving only the quadriceps muscles). In addition, training-induced structural changes in muscles occur later in time than adaptations in metabolic properties or peripheral circulation.[22]

Limits of stability

Our results demonstrated significant improvements in balancing ability with respect to endpoint excursion (EPE), maximum excursion (MXE), and directional control (DCL) after cycling intervention. But there was no time-by-group interaction for these parameters. These findings would suggest that an

Table 2. Effects of Training Program on Physiologic Parameters and Indices of Limits of Stability

Parameters and Indices	Group	n	Baseline	Post-test	Time (pre vs post)	Time × Group Interaction
MVC force (lb) Sound knee extensors	FES-LCE	8	42.54 ± 9.81	40.79 ± 9.96	.073	.731
	LCE	8	51.79 ± 8.20	49.04 ± 6.74		
MVC force (lb) Paretic knee extensors	FES-LCE	8	28.17 ± 12.00	29.17 ± 12.49	.456	.663
	LCE	8	28.33 ± 12.01	28.50 ± 13.23		
RT (s)	FES-LCE	8	2.01 ± 1.93	1.43 ± 0.79	.321	.873
	LCE	8	1.33 ± 0.55	0.87 ± 0.30		
MVL (deg/s)	FES-LCE	8	1.89 ± 1.05	2.73 ± 1.87	.291	.254
	LCE	8	2.15 ± 1.15	2.24 ± 1.01		
EPE (% of LOS)	FES-LCE	8	39.75 ± 21.00	49.75 ± 22.49	.031†	.822
	LCE	8	31.38 ± 9.16	42.88 ± 13.17		
MXE (% of LOS)	FES-LCE	8	49.75 ± 22.71	63.13 ± 19.41	.000‡	.388
	LCE	8	43.00 ± 11.92	61.13 ± 8.90		
DCL (% of accuracy)	FES-LCE	8	77.50 ± 8.26	84.38 ± 4.27	.013†	.834
	LCE	8	69.38 ± 12.21	77.13 ± 12.37		

Note. Values are mean ± SD
†$P < .05$.
‡$P < .01$.

enlargement of subject's COG excursion toward the paretic side target, linked to an improved standing postural stability control accompanying the enhanced skeletalmuscular afferent feedback and neuromuscular coordination after exercise. Moreover, Janssen et al.[21] reported that the effects of cycling exercise can lead to improvements in functional performance (i.e., scores on the Berg Balance Scale increased by 6.9% and the six-minute walk test improved by 14.5%). Additionally, this improved functional performance may be essential to maintain physical independence and to reduce the risk of falling.[23,24]

Functional electrical stimulation

Application of FES for the purpose of increasing additional muscle contraction has been well documented. But in our investigation, there was no apparent difference for any measured variable between both groups. This may be caused by eliciting muscle force output with low stimulation intensities that can avoid the uncomfortable or painful sensations during leg cycling intervention. Hence, the measured variables including muscle strength and balancing ability were likely not related to the use of FES because eliciting intensities were respectively low.

Study limitations

There are limitations in this study such as short duration of the treatment and lack of long term follow up data. Future studies are needed to explore the long-term effects of this intervention on motor recovery and ambulation in patients with stroke.

CONCLUSION

A functional electrical stimulation facilitation program integrated into cycle ergometer activities can significantly enhance the balancing ability in patients

recovering from stroke. However, the paretic leg was not affected directly and apparently after FES-cycling training than the LCE group. We concluded that the application of FES had no additional beneficial effects in this experimental group of subjects with stroke.

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功能性電刺激輔助踩車訓練對於中風患者下肢肌肉力量與站立平衡之影響

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目的：評估中風患者使用功能性電刺激輔助踩車運動訓練前後，偏癱下肢肌肉力量及站立平衡反應，是否會有不同之變化。

方法：利用隨機分配方式將十六位受測者分為電刺激踩車實驗組（八位）與踩車訓練控制組（八位）。在進行踩車運動前，病患均先接受偏癱下肢肌肉力量與站立穩定極限平衡的評估（分別以手握肌力測試儀及電腦動態平衡反應測試儀來量化）；而當兩組受測者分別完成踩車運動後，再次評估其偏癱下肢肌肉力量與穩定極限平衡的變化。

結果：兩組受測者其兩側膝伸直肌肌力於踩車訓練前後並無明顯統計差異；但在往偏癱側的站立穩定極限平衡測試參數則發現：重心初次位移距離（$P = .031$）、重心最大位移距離（$P = .000$）與重心位移方向控制（$P = .013$）有明顯的增加改善趨勢。而兩組間的踩車運動訓練對於中風患者之膝伸直肌肌力，以及站立穩定極限平衡測試指標等皆無明顯之差異影響。

結論：本研究可以證實並觀察出，兩組受測者在進行踩車運動訓練後，皆明顯有助於改善增加偏癱側的站立穩定極限平衡反應表現，其中包含了重心初次位移距離、重心最大位移距離及重心位移方向控制等參數。但是在兩組間應變數的比較上，功能性電刺激針對於踩車實驗組而言，卻無法額外增加中風患者膝伸直肌肌力與站立穩定極限平衡反應表現。未來的臨床研究希望可以徵召較多的受試者，參與探討有關功能性電刺激輔助踩車訓練後，對於功能性動作回復與步態行走之長效性影響。

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關鍵詞：功能性電刺激、踩車運動、肌肉力量、站立平衡、腦中風

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Successful Treatment of Olfactory Neuroblastoma with IMRT: a Case Report

Chi-Yuan Yeh

Olfactory neuroblastoma(ONB) is a rare and aggressive tumor of the olfactory mucosa that has been traditionally treated with radical surgical excision. Nevertheless, these rare tumors are still associated with high rate of recurrence and mortality. Here in, I report a novel therapeutic approach using intensity modulated radiotherapy (IMRT).

IMRT in conjunction with weekly cisplatin should be considered in patients with unresectable olfactory neuroblastoma since this technique can deliver an adequate tumoricidal dose. Careful IMRT treatment planning is necessary to avoid or minimize untoward side effect.
(Tungs' Med J 2009; 3: 91-97)

Key words: olfactory neuroblastoma, IMRT, radiotherapy

INTRODUCTION

Olfactory neuroblastoma (ONB) is a slow growing malignant tumor originated from the olfactory mucosa, and is also known as esthesioneuroblastoma. It is a rare tumor of the nasal cavity which is neuroectodermal in origin. This tumor was first described in 1924 by Berger as a tumor of the olfactory mucous membrane with 2 distinct attributes: (1) true neuroepithelial rosettes, and (2) undifferentiated masses of nuclei and cords of fibrils[2].

Olfactory neuroblastomas usually arise in the nasal fossa from the olfactory region, the roof of the nasal cavity, the anterior and middle ethmoid regions, the superior turbinate, and the superior portion of the nasal septum[3]. Obert et al[4]. described them as a primary nasal neurogenic tumors originating from the olfactory mucous membrane, while other site of origin have been speculated, such as the sphenopalatine ganglion, organ of Jacobson, on the inferior aspect of the nasal septum, and the ganglion of Loci[4]. It usually afflicts the patient in the second or third decade of life with a history of chronic nasal obstruction, epistaxis, rhinorrhea and anosmia.

Obert et al[4]. treated 7 patients of ONB with electrocoagulation, surgery and radium implants, achieving median local control duration of only 1 year. Treatment modalities have included external radiation alone, combined radiation and craniofacial resection. More advanced lesions are treated with 3D conformal radiotherapy. Chemotherapy is unproven and has been reserved for recurrence or distant metastatic disease[5]. Here we present a case of olfactory neuroblastoma that was successfully treated with intensity modulated radiotherapy (IMRT).

Case report and treatment protocol

The patient is a 67 year old male construction worker who was referred to the radiation oncology department due to a complaint of right cheek pain

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associated with swelling of 1 month's duration. The pain was progressive and radiation to right ear. The patient also complained of a recurrent toothache of right upper molar. The patient visited a dentist and dental caries was diagnosed. The lesioned tooth was extracted promptly but right cheek pain didn't resolve. CT scan was performed at May 13, 2004 finally and revealed a large enhanced tumor in right maxillary sinus with bony destruction of right frontal, ethmoid and sphenoid sinuses and orbital cavity (Fig.1). Pathology of the tumor revealed olfactory neuroblastoma, the neuron specific enolase stain was positive. CK, S100, L26, MT1, LCA and desmin stain were all negative. The histologic section of the tumor showed solid sheets of small, uniform malignant cells with scanty cytoplasm round nuclei and indistinct nuclear membrane.

Physical examination of the patient revealed slight swelling over right maxilla, slight edema over right upper eyelid. Oral examination revealed a fixed protruding mass on the right upper molar area, with extension to the hard palate. Sinoscopy performed on May 15, 2004 revealed bulging over medial wall of the right maxillary sinus with bony erosion; the friable grayish tumor was noted to fill the entire ethmoid sinus and maxillary antrum. There was no visual defect and cranial nerve palsy even the tumor was ob-

Table 1. Dulguerov, Allal, and Calcaterra staging system for ONB[6]

stage	Tumor involvement
T1	Tumor involving the nasal cavity and/or paranasal sinus(excluding sphenoid), sparing the most superior ethmoidal cells
T2	Tumour involving the nasal cavity and/or paranasal sinuses (including the sphenoid) with extension to or erosion of the cribriform plate
T3	Tumour extending into the orbit or protruding into the anterior cranial fossa, without dural invasion
T4	Tumour involving the brain
N0	No cervical lymph-node metastasis
N1	Any form of cervical lymph-node metastasis
M0	No metastases
M1	Distant metastasis

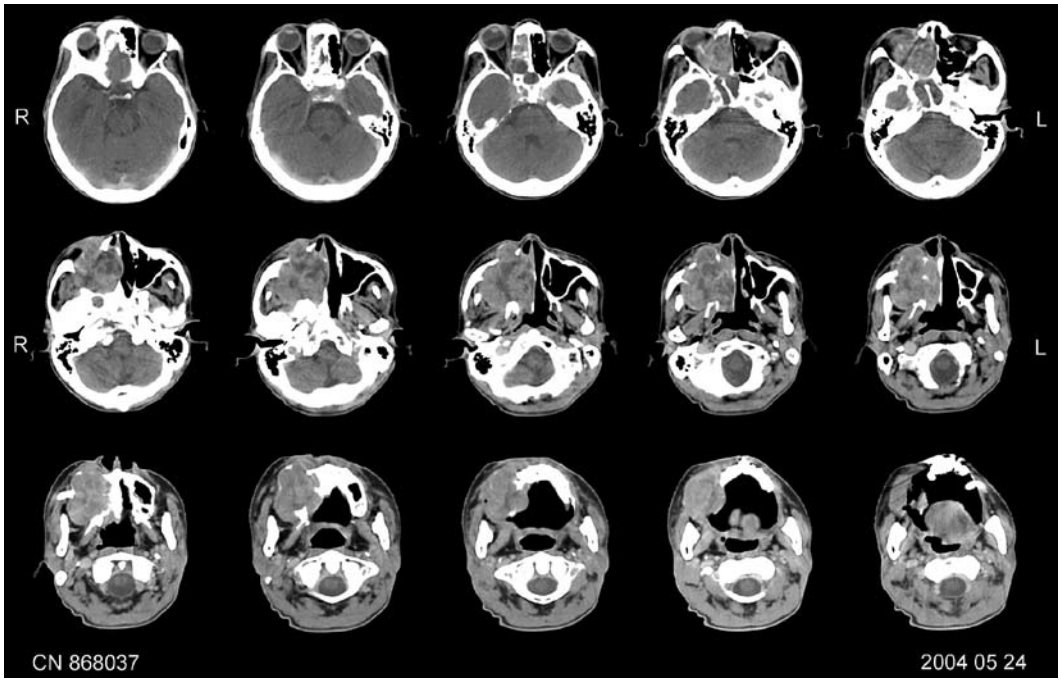

Fig. 1 Axial CT scan of the patient before IMRT

served to encompass the right optic nerve in CT scan (Fig.1). Palpation of the patient's neck did not reveal any lympadenopathy. Systemic review of the patient did not reveal any evidence of distant metastasis.

This patient was classified as stage C based on the earlier Kadish system[9] and T3N0M0 based on the Dulgerov system. The patient was treated with intensity modulated radiotherapy (IMRT) for a total dose of 6300 cGy (June 30, 2004 to August 20, 2004) concurrently with weekly cisplatin iv at 30 mg/m^2. However, the patient refused further chemotherapy after 2 courses of cisplatin due to severe nausea and vomiting.

The entire tumor volume was counted to be 241.78 cm^3. Intensity modulated radiotherapy was delivered using the step-and-shoot technique. Seven coplanar beams were used in our treatment with energy of 6 MV for each beams. The gantry angles for the 7 beams were 0°, 45°, 90°, 120°, 160°, 200°, 280° (Fig.2).

RESULTS

The primary tumor was covered with the 95% isodose curve. Repeat CT scan on Sept. 9, 2004 revealed a residual enhanced tumor over right maxillary sinus with bony invasion. Surgical resection of the residual tumor was not recommended by ENT doctor due to danger of the delayed wound healing and possible bleeding since high dose of IMRT has been given earlier. After consultation with the radiologist and ENT specialist, we concurred that viable tumor left was the culprit for the persistent pain in the right maxillary area. A very small field IMRT boost 58 days after the last day of radiotherapy was arranged, a total dose of 3300 cGy was given from Oct. 1, 2004 to Nov 4, 2004. The CTV or clinical target volume showed a less than adequate 100% coverage from 4000 cGy to 8000 cGy dose range due to the fact that we had to restrict the dose to the right eye and right optic nerve.

The patient completed the IMRT without treatment interruption in between. The patient experienced radiation induced oral mucositis and dermatitis of the right maxillary area during treatment. The patient was followed up every month after radiotherapy, then he was admitted from January 12 to 14, 2006 due to right nasal synechia and right lacrimal duct obstruction, this was considered to

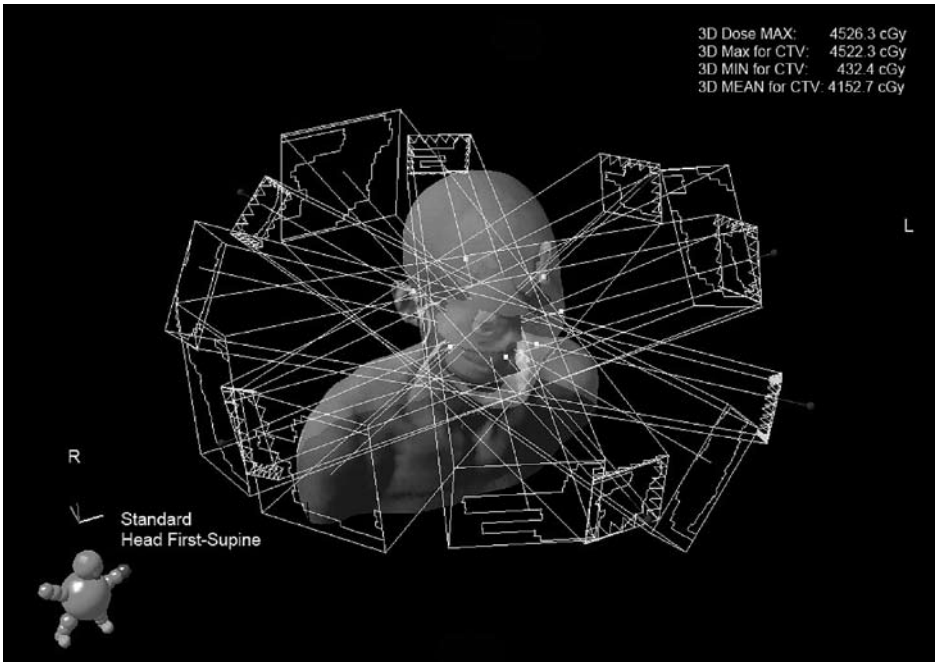

Fig. 2 Gantry angle of the 7 beams used for IMRT

be induced by radiation. The patient was noted to have persistent tearing and redness of right eye, no visual disorder was noted despite right optic nerve had received high radiation dose. CO_2 laser lysis of synechia, dacryolacrimal duct resection and tube insertion was performed. The patient's tearing and redness of the right eye was relieved by the operation. The patient visual acuity test was 0.2 of both eyes 26 months after the last radiotherapy.

An MRI of nasopharynx with contrast was performed at April 15, 2006, the findings included (Fig.3):

1. Abnormal signal intensity involved right ethmoid, maxillary wall & sphenoid sinuses but not enhanced.
2. Non visualization of the right maxillary bone.
3. Non visualization of the right superior nasal turbinate, decreased right ethmoidal air cells.
4. A small 1.4 cm abnormal signal (non- enhanced) lesion adjacent to the right inferior side of the right sphenoid sinus.

The impression was a nonspecific abnormality of the right ethmoid and sphenoid sinus without evidence of local recurrence.

There was no tumor recurrence or distant metastasis noted at the last follow-up, that was 29 months after last radiotherapy. The patient still lives a normal and independent life.

DISCUSSION

This is a case of advanced stage olfactory neuroblastoma of the right maxillary sinus treated successfully with IMRT. Uncertainty about the precise histological origin has led to the use of various names for this tumor; two terms that has gained wide acceptance in recent publications are esthesioneuroblastoma and olfactory neuroblastoma (ONB). There are many opinions about its origin, diagnosis, and management. Three other factors contribute to the controversy. First, the tumor shows varying biological activity, ranging from indolent growth, with

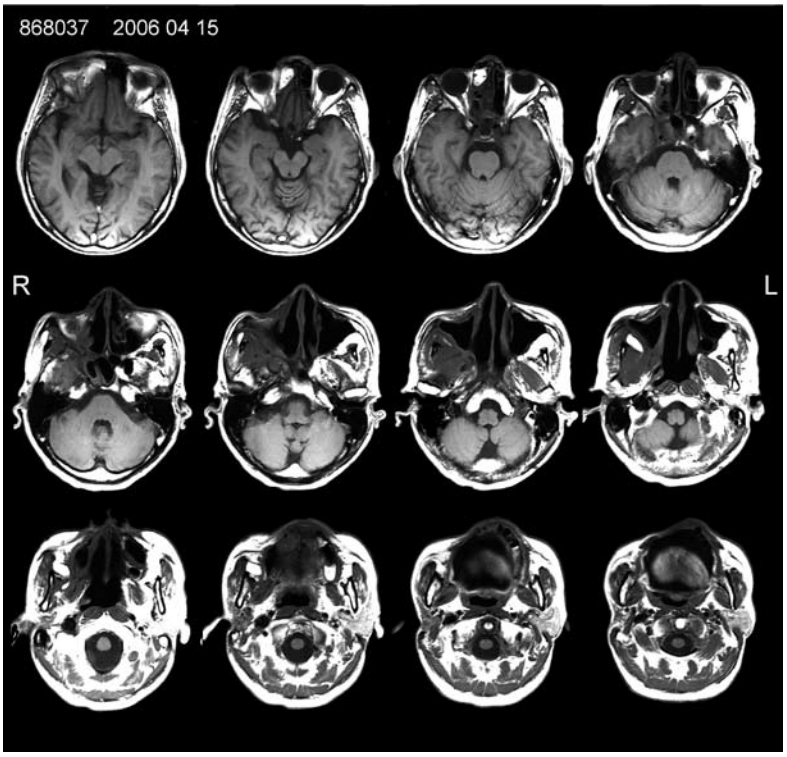

Fig. 3 MRI of the patient 18 months after IMRT showing complete tumor regression

patients surviving with known tumor for more than 20 years, to a highly aggressive neoplasm, capable of rapid widespread metastasis with survival limited to a few months. Second, ENB is usually confused with other undifferentiated neoplasm of the nasal cavity. Third, no universally accepted staging system is available [4,7].

Standard treatment for ONB included en-bloc surgical intervention, radiotherapy and chemotherapy. Surgery has been preferred as the initial treatment modality, followed by radiotherapy[4,7]. Broich et al.[5], in a literature review of ONB cases, noted that 43.21%(388 cases) had received a combination of surgery and radiotherapy, 25.17%(226 cases) were treated by surgery alone while 18.37%(165 cases) by radiotherapy alone. They also reported a local recurrence of 59% for surgery alone, while postoperative radiotherapy could reduce local recurrence rate to 12.5%. Chemotherapy alone or in combination with other treatment was given in 13.2% (119 cases), while 11 patients received a bone marrow transplant. The mortality rate was the highest for radiotherapy alone (30.7%) compared with surgery and adjunctive radiotherapy (18.75%) and surgery alone (12.50%). They concluded that the best choice of treatment was wide surgical resection followed by radiotherapy and surgery alone was indicated when the stage T1 patient with a small tumor located well below the cribifrom plate [7]. Invasion of optic chiasma, cavernous sinus and/or middle fossa, as well as the presence of distant metastases excludes radical surgery as seen in our patient, while invasion of the orbit or maxillary sinus are not [5].

Berger et al.[2] was the first to report using radiotherapy in the treatment of ONB. Radiotherapy is indicated for those patients who had residual disease after resection or unresectable tumors. Conventional radiotherapy utilizes external megavoltage beams using a three-field technique arranged in an anterior port combined with wedge bilateral field configuration. The recommend dose ranged from 55 Gy to 65 Gy[4,7,8]. Arafat et al.[1] used IMRT to treat ONB with curative intent in Kadish stage B(4 patients) and C (4 patients) after surgical resection. Mean IMRT doses delivered to CTV1 (gross tumor volume and surgical resection bed,) and CTV2 (adjacent at-risk tissues and nodal levels.) were 62.2±0.8Gy and 58.6±1.1 Gy, respectively. Mean total CTV coverage with target doses was 96.3%±1.7. Mean optic chiasm, optic nerve, eye, lens, and temporal lobe doses were 38.6±6.1 Gy, 48±5.5 Gy, 23±4.4 Gy, 10.5±3.6 Gy, and 21.1 ±6.6 Gy, respectively. All patients remain free of disease progression and have no severe late radiation morbidity after a mean follow-up duration of 25 months (range:12-42).

Obert et al. [10] had emphasized that radiotherapy alone is not adequate for these type of tumor, albeit he was using radium implant and superficial x-rays for treatment, he noted that other treatment modality should be included. Recently chemotherapy has entered the treatment protocol for ONB. The agents most frequently used are cisplatin, etoposide, adriamycin, cyclophosphamide, vincristin, 5-fluorouracil, doxorubicin and thiothepa. Chemotherapy should be confined to inoperable lesions or large, otherwise untreatable, recurrences[7]. Eich et al.[11] treated 6-year old boy with good result. Combination chemotherapy was given (2 cycles of adriamycin, cisplatin, vincristine, DTIC, 2 cycles of procarbazin, cisplatin, CCNU and 3 cycles of ifosphamide, VP- 16) preoperatively. After incomplete surgery he was irradiated with a total dose of 4400 cGy. After a follow-up period of 155 months the patient is alive with no evidence of disease.

Gender, tobacco and, alcohol usage, presentation with regional disease, margin status at time of initial resection, and treatment variations did not reach prognostic significance with regards to recurrence or survival[9]. A separate study showed that the presence of palpable lymph nodes as an important prognostic factors for survival (29% with nodes vs 64% without nodes[10]). In addition, The Kadish staging can also predict the patient's outcome[6, 7, 9,10,12]. The mean 5-year survival for stage A, B, and C was 72%, 59% and 47% respectively[6].

Treatment complications in patients with esthesioneuroblastoma remain high. This is, at least in part, reflective of aggressiveness and difficulty in the management of this disease, and because of the location of esthesioneuroblastoma, it is difficult to deliver an adequate dose of radiation without exceeding the tolerance dose of the critical structures such as the brain, optic chiasm, optic nerves, and orbits. Our patient experienced limited visual acuity of 0.2 for both eyes 26 months after radiotherapy , this being most likely to be due to radiation induced

optic neuropathy. Thus, we have to include the right optic nerve in high dose treatment volume since the tumor is actually invading the right orbital cavity (Fig.1). Radiation-induced optic neuropathy (RION) is caused by ischemia of the optic nerve. Occlusive obstruction of the arteries supplying the optic nerve head and the retrolaminar part of the optic nerve, including the optic chiasm leads to optic atrophy.

The tolerance dose (TD_{50}) with a 50% chance of complication happening in 5 years for the optic nerve and chiasma is 7200 cGy[5]. Bhandare et al.[5] in a study of 272 patients with irradiation to the optic nerves and optic chiasm reported that the 5 year rate of freedom from RION was 95% for <6300 cGy group and 78% for > 6300 cGy group. The median and mean optic nerve dose in their patients who developed RION was 6800 cGy and 6700 cGy respectively[5]. Treatment for RION including hyperbaric oxygen, corticosteroids, anticoagulants and optic nerve sheath fenestration had produced mixed results [5].

CONCLUSION

This is the first reported case of ONB successfully treated with IMRT in Taiwan. Dose escalation with IMRT for this tumor is a feasible method of treatment since it is possible to avoid radiation induced toxicity to the normal surrounding structures such as the eyes, parotid glands, and optic nerves while achieving a tumoricidal dose to the primary tumor, further accumulation of patients treated with IMRT is needed to gain more experience with this newer modality of treatment.

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嗅神經母細胞瘤：強度調控放射治療經驗

葉啟源

嗅神經母細胞瘤（olfactory neuroblastoma; ONB）是一種來自於嗅黏膜的惡性腫瘤，也是在鼻腔中少見的腫瘤，傳統的治療方式是以手術進行切除，然而常伴隨著高復發率及高致死率。本案例是以強度調控式放射治療技術（Intensity Modulated Radiation Therapy; IMRT）治療 ONB 的報告。

對於 ONB 無法開刀的病患，IMRT 合併每週 cisplatin 化學治療是另一種可以選擇的治療方式。因為 IMRT 可以給予腫瘤最大的放射線劑量，並保護正常的組織，可以將副作用減至最低。然而；不幸的是在治療的過程中因視神經受到過高輻射劑量照射而對病患視力造成損傷。因此雖然 IMRT 的治療計畫可以減少正常組織接受的劑量，還是要注意所有可能引起的傷害以減輕正常器官的副作用。

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關鍵詞：嗅神經母細胞瘤、強度調控放射線治療、 放射線治療

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Successful Pacemaker Lead Implantation in Severe Subclavian Vein Stenosis

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Herein, we report a case of an 84-year-old man with atrioventricular (AV) conduction defect, needed for dual-chamber pacemaker (DDDR) implantation. During the procedure, there was difficulty encountered in advancing the pacemaker lead. Venography showed severe stenosis over the proximal part of the left subclavian vein. Balloon angioplasty was done which subsequently lead to successful pacemaker implantation. Adequate management for the dilemma of the lead placement via a stenotic subclavian vein is still controversial. However, we find the balloon angioplasty can be an acceptable alternative method in this setting.
(Tungs' Med J 2009; 3: 98-102)

Key words: subclavian vein stenosis, pacemaker implantation

INTRODUCTION

Pacemaker implantation is greatly beneficial for patients who have symptomatic sinus node or atrioventricular (AV) node diseases. One important technique requires a direct-visualization of the cephalic vein via surgical cut-down which can lower the complication of blind subclavian vein (SV) puncture. However, severe SV stenosis may be encountered occasionally which makes advancement of pacemaker lead difficult. Percutaneous transluminal angioplasty (PTA) of SV is relatively safe and maybe an effective procedure in this setting.

CASE REPORT

This is a case of an 84-year-old male who presented with frequent dizziness and several episodes of near syncope for about 3 months prior to his consultation. He also experienced generalized weakness and easy fatigability after a short walk or after taking a flight of stairs. He is hypertensive and had history of pneumoconiosis requiring lung volume reduction. Upon admission to our institution, he had severe bradycardia with cardiac rate of 30-40 beats per minute. Electrocardiography showed Mobitz type II second degree AV block with right bundle branch block. Echocardiography revealed concentric left ventricular (LV) hypertrophy with good LV function. Ejection fraction (EF) was 76%. There was aortic dilatation measured at 41mm. Mild tricuspid regurgitation (pressure gradient = 36.2mm Hg) was also noted. Pre-operative chest x - ray showed hyperinflation with chronic infiltration, pulmonary miliary lesions in both lung and post-lobectomy operation change in left upper lobe.

The venous entry of pacemaker lead was via cephalic vein located in the left delto-pectoral groove. Surgical cut down of the vein under direct-visualization was done. After which, the ventricular lead

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(VL) (Medtronic CapSure Fix Novus 5076-58) was advanced barely enough and there was resistance encountered, however. Following by the common-sized guiding sheath (7 Fr. Peel-away sheath with detachable hemostasis valve) insertion which failed to pass through the left SV under a high resistance. And then, a long sheath (7 Fr., Medtronic 6207 BTKL1) was also tried but failed to advance. The thin hydrophilic wire reached the right atrium but it provided poor support for the introducer sheath. Then, we proceeded to do contrast injection via the long sheath from distal part of left SV. Venography showed near-total and concentric stenosis (Figure 1.A) of the proximal SV with an inner-diameter stenosis (DS) of 85%. Measured minimum lumen diameter (MLD) was 0.76mm and reference diameter (RD) was 5.37mm. Collateral was seen around the shoulder and the upper chest.

PTA for endovascular angioplasty was done via 6F sheath and using balloon catheter, 5.0 x 40 mm (Wonda, Boston- Scientific, Ireland) (Figure 1.B) to dilate the tight lumen stricture of the SV. Post-angioplasty venography showed a DS of 67%, MLD of 1.94mm, and RD of 5.99mm (Figure 1.C). Thereafter, successful passage of atrial lead (AL) (Medtronic CapSure Fix Novus 5076-52) was possible (Figure 2). Pacing threshold was 1.25 Volt at 0.4 ms (AL) and 0.5 Volt at 0.4 ms (VL). Pacing impedance was 643 Ω (AL) and 618 Ω (VL). Intrinsic ventricular de polarizations was absent during post-operative follow-up. The patient tolerated the procedure well, with no complications noted. After one-month follow-up, there was no edema noted over head, arm and left neck vein. Pacemaker function is also good.

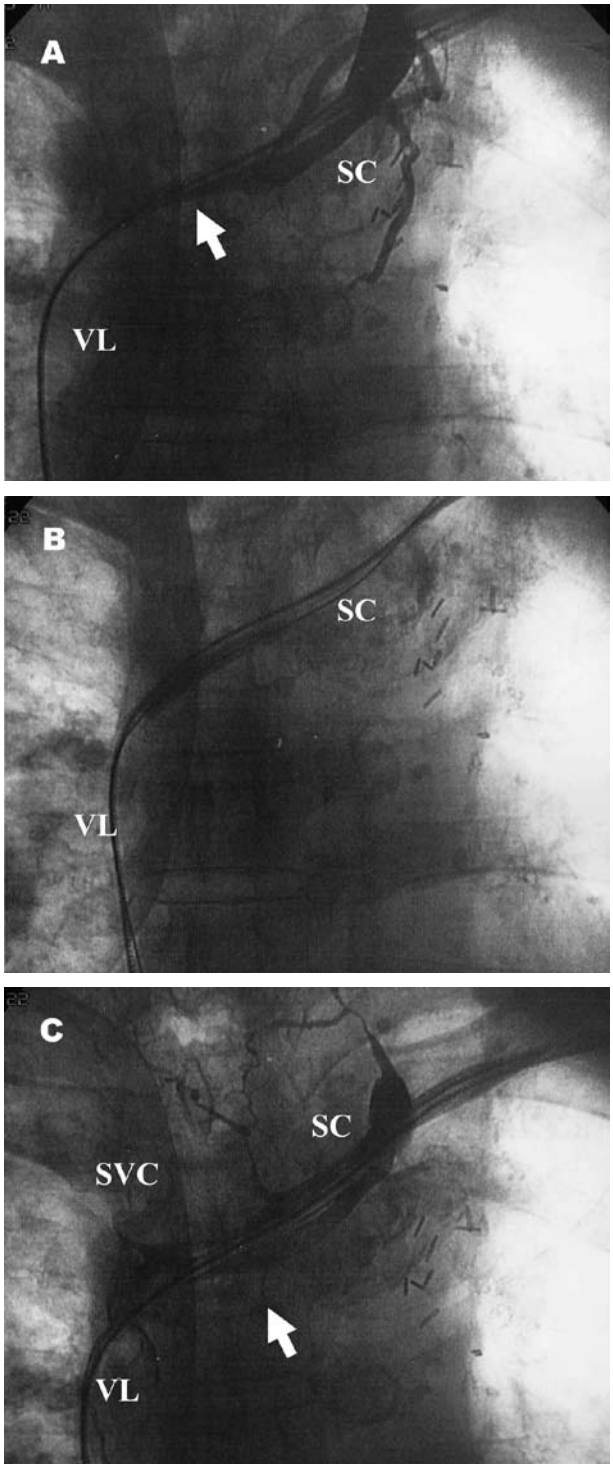

Fig. 1 Left subclavian venography, before dilataion (A), during balloon dilatation (B) and after dilatation(C). SC = subclavian vein; SVC = superior vena cava; VL = ventricular lead.

DISCUSSION

The prevalence of SV stenosis before pacemaker implantation is not common. Nevertheless, once the pacemaker is implanted it may make the SV stenosis worsen causing clinical symptoms. Usually SV stenosis or occlusion can occur in about 30 to 50% of hemodialysis patients receiving temporary catheterization. The patient we reported here did not have hemodialysis, central vein catheterization and left neck, arm swelling. Because of being clinically asymptomatic with high-degree SV stenosis and abundant collateral circulation before the operation, we decided to combine additional balloon angioplasty to maintain the access smooth in order to facilitate electrode

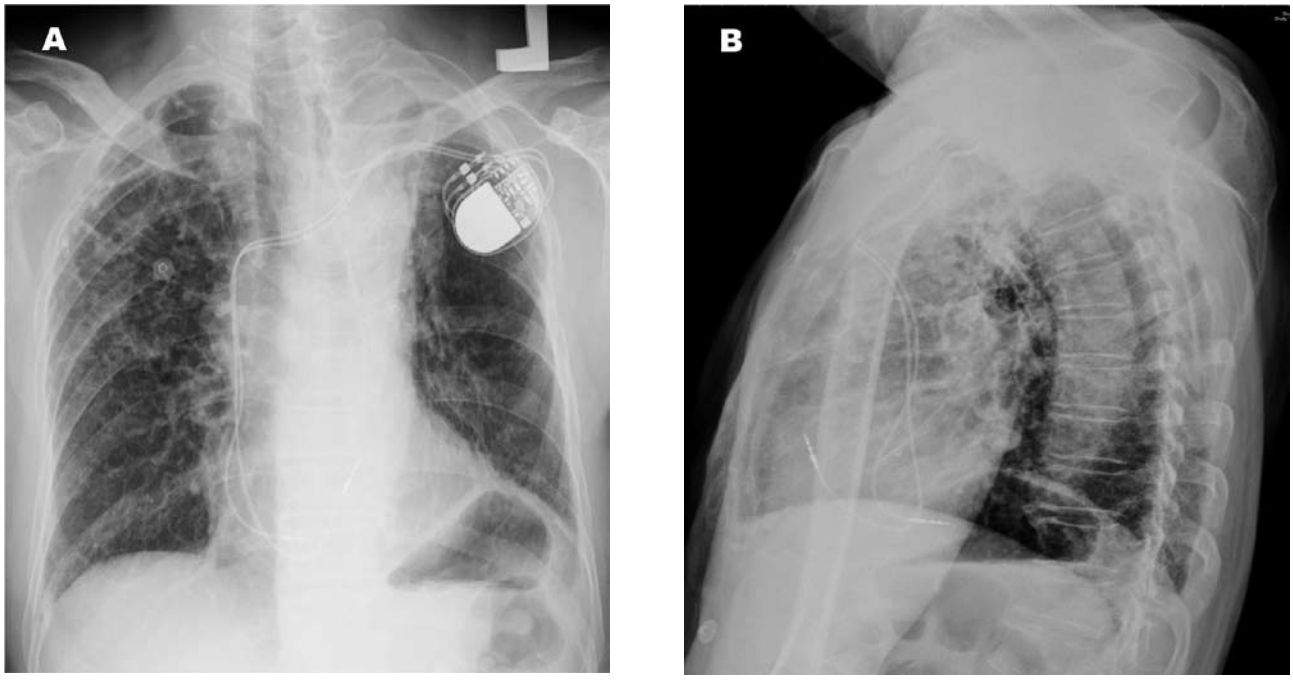

Fig. 2 Postoperative chest x-ray in posterior-anterior (A) and lateral (B) view.

placement.

Additional PTA or balloon angioplasty is safe and acceptable for the purpose of expanding the inner diameter of subclavian vein immediately and place the electrode lead smoothly. Another alternative is PTA plus stenting to secure the venous route. However, stenting provided more protective effect to hemodialysis patients with central vein stenosis or obstruction, but was not certain for others[1]. Stenting-associated potential damage of pacemaker lead was a concern. Although published reports showed that stenting did not affect the lead function such as pacing threshold, impedance and noisy- signal. The reason behind this may be that the pacemaker wires are covered by a thick coat of insulation that usually consists of silicone rubber or polyurethane.

In the study of Korkeila and his colleague[2], approximately 14% of venous obstruction occurred at 6 months after pacemaker implantation. Minimal catheter movements against the vein wall can result in chronic endothelial damage which is possibly enhance by thrombophlebitic reactions due to catheter-adherent fibrin sheaths and biofilms[1]. Theoretically, permanent pacemakers can cause an initial endothelial injury at the point of insertion of the pacemaker into the SV, and the wire-associated mechanical stress may result in vessel wall inflammation, excessive proliferation of fibrous tissue, and thrombus formation around the lead. However, post operative complication of pacemaker implantation related with SVC or SV syndrome and lead-related venous stenosis or occlusion were still relatively asymptomatic and rare[3,4]. Serious thrombosis and embolic complications are reported to occur in < 3% of cases[5,6]. Most obstructions occurred at the same site where the narrowest site of vein is at baseline. Poor left ventricular function (LVEF $\leqq$ 40%) was also considered as a significant risk factor of venous stenosis[7]. Once SV became occlusive and symptomatic that may mostly improve with time as collateral circulation develops. The patient had had much venous collateral perfusion before the procedure of angioplasty and electrode lead placement.

Kolb and his colleague[8] had reported using a very thin pacing lead was possible to pass successfully though the high degree stenotic access. But preparing each brand of thin pacing leads is not always easy unless performing SV venography beforehand.

Simple venography may be necessary routinely for each patient to establish patency of the access and to delineate a clear-cut image of central venous tree.

Right-sided (contralateral) venous access is another approach in the setting of left SV stenosis, but would require the need to create a new tunnel which can add to patient's discomfort coping with two surgical wounds. Incidence of infection may also be increased. Routine central venography via peripheral vein maybe done to guide us to select an appropriate side for implantation and may prevent dealing with a SV stenosis.

In some cases, adequate balloon dilatation is helpful to pass the electrodes for the stenotic vessel, especially in clinical asymptomatic patients. It is safe and does not affect pacemaker electrodes function (one-month follow-up VL impedance 545 Ω and pacing threshold: 0.75volt with 0.4 ms). However, there are currently no general recommendations in the management of SV stenosis when encountered during pacemaker implantation owing to very limited data are available from the literature.

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心律調節器導線成功在嚴重下鎖骨靜脈狹窄置放

劉人福[1,2*]　張伯丞[3]

84 歲男性罹患心房心室傳導缺失，須放置雙腔心律調節器，於第二條心房導線置放手術過程中發生困難，當時執行靜脈血管攝影，即時呈現左下鎖骨靜脈近端嚴重狹窄導致無法放入，因而執行氣球擴張術來擴張靜脈狹窄處，之後順利放置心房導線完成雙腔心律調節器置放，目前對於心律調節器導線於下鎖骨靜脈狹窄時處置仍有爭議，立即的氣球擴張術是可以被接受的方法。

（童綜合醫誌 2009; 3: 98-102）

關鍵詞：下鎖骨靜脈狹窄、心律調節器導線置放

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Unusual Delay of Computerized Tomography Contrast Medium Imaging in a Heart Failure Case

Yi-Lun Tsai[1], Shen-Chau Huang[2]*

On computed tomography (CT), a global delay in left ventricular and aortic enhancement, distention of the inferior vena cava (IVC), hepatomegaly, and reflux of contrast medium into the superior vena cava (SVC), IVC and hepatic veins may aid the physician on the diagnosis of congestive heart failure. These abnormal enhancement patterns are thought to reflect the slowing of blood flow and increased passive venous pressure secondary to low cardiac output.
(Tungs' Med J 2009; 3: 103-106)

Key words: Computed tomography (CT), delayed left ventricular enhancement, reflux of contrast medium into hepatic vein, congestive heart failure

INTRODUCTION

In usual protocol of chest CT scans, the aortic enhancement is noted grossly within 30 seconds after contrast medium injection in patients with normal cardiac function. Reduction of cardiac output results in a substantial increase the time to peak aortic enhancement[1]. The diagnosis of congestive heart failure on CT scans may be established in several image manifestations including delayed left ventricular enhancement, distention of the IVC, hepatomegaly, and reflux of contrast medium into the SVC, IVC and hepatic veins. These are caused by slower blood flow and increased passive venous pressure, which are secondary to low cardiac output[2,3].

CASE REPORT

A 45-year-old previously healthy man presented to the emergency department with complaints of dyspnea and chest tightness. He developed progressive orthopnea and bilateral leg edema after an episode of upper airway infection about one month ago.

On admission, his blood pressure was 136/100 mmHg. Physical examination revealed irregular heart beat and bilateral leg pitting edema. Complete ECG showed atrial fibrillation with a ventricular rate of 174/min. Laboratory tests showed normal value except mildly impaired renal function (BUN 22.6 mg/dL, Creatinine 1.9 mg/dL).

Contrast-enhanced chest CT scans were arranged to rule out acute aortic dissection, with the first scan obtained at 45-second delay after contrast medium injection from left antecubital vein through left brachiocephalic vein, which is beyond the temporal window for assessing aorta in our protocol. It demonstrated strong enhancement of right ventricle (Figure 1), abnormal enhancement of SVC(Figure 2), IVC, hepatic veins (Figure 3), merely mild enhancement of left ventricle left atrium and no bright up at aorta (Figure 1). The second scan obtained with 160-second delay after contrast medium injec-

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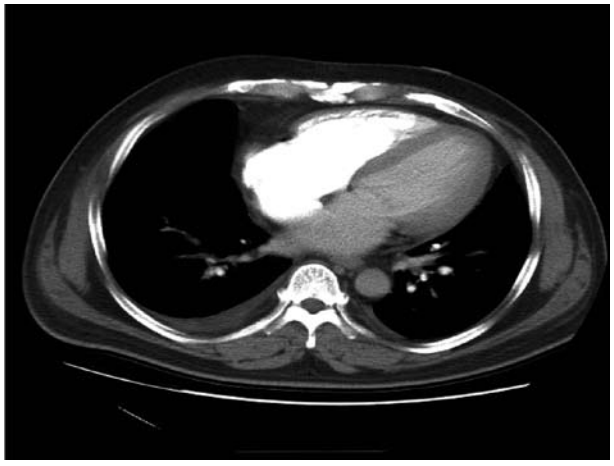

Fig. 1 The first scan obtained at 45-second delay after contrast medium injection, which demonstrated strong enhancement of right ventricle with merely mild enhancement of left ventricle and left atrium, and no bright up at aorta.

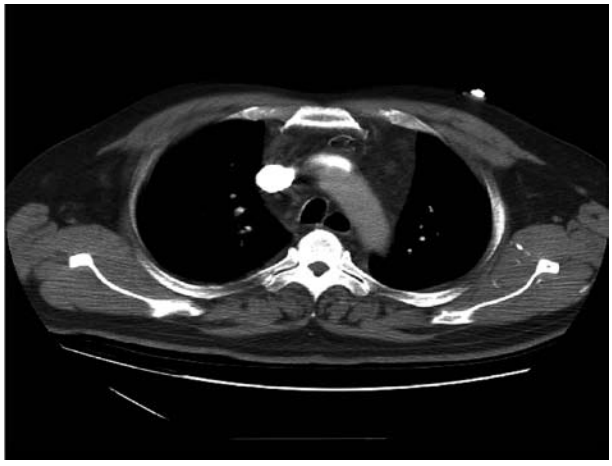

Fig. 2 The same image series as that of figure 1 showed abnormal enhancement of SVC.

tion showed strong enhancement of the four cardiac chambers. Bed-side-echocardiography revealed dilated left atrium and ventricle, poor LV contractility with ejection fraction of 19%. Under the diagnosis of dilated cardiomyopathy with congestive heart failure, the patient received oxygen, digitalis, nitroglycerin and diuretics therapy. The symptoms subsided gradually and he was discharged with stable condition two weeks later.

DISCUSSION

There are many factors influencing the time to peak aortic enhancement on CT including patient weight, venous injection site, kinds, concentration, injection volume, injection rate of the contrast medium[1,2,3,4,5]. However, according to literatures that the time to peak aortic enhancement is within 45 seconds after contrast medium injection[2,3]. Reduction of cardiac output results in a substantial increase in the time to peak aortic enhancement[6].

The patient presented signs of heart failure at the emergency department, so we have delayed the timing of post-contrast scan. The initial CT scan was obtained with 45-second delay after contrast medium injection. The timing is beyond our protocol but still within the temporal window for assessing aorta. However, the images still revealed nearly no enhancement at aorta. The second scan was obtained

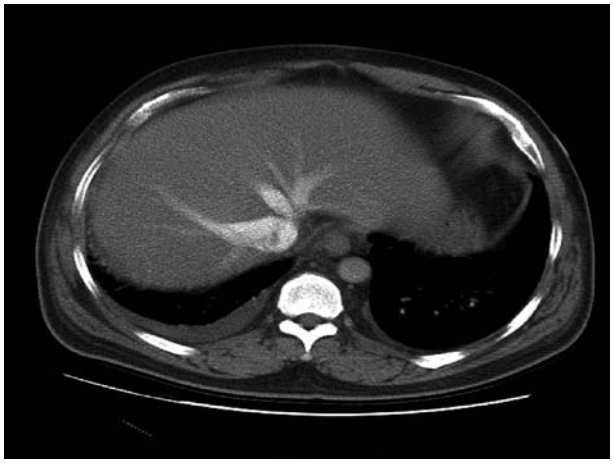

Fig. 3 The same image series as that of figure 1 and 2 showed reflux of contrast medium into the perihepatic IVC and hepatic veins.

with 160-second delay after contrast medium injection, indicating the timing is far more beyond the temporal window of aortic enhancement in the patients with normal cardiac function.

Abnormal patterns of contrast medium enhancement such as a global delay in left ventricular, aortic enhancement, reflux of contrast medium into the SVC, IVC and hepatic veins may aid the physician on the diagnosis of congestive heart failure on computed tomography (CT)[7, 8]. In addition, other abnormal image manifestations such as distention of IVC and

hepatomegaly may also be suggestive of the diagnosis of heart failure[7, 8]. These abnormal enhancement patterns are thought to reflect the slowing of blood flow and increased passive venous pressure secondary to low cardiac output.

Other factors such as cardiac arrest or pulmonary hypertension may also result in the abnormal enhancement patterns including global delay in left ventricular, aortic enhancement and reflux of contrast medium into the SVC, IVC. However, these factors are not found in our patient.

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一例心臟衰竭患者電腦斷層影像中的不尋常延遲顯影現象

蔡依倫[1]　黃聖超[2*]

在電腦斷層影像中，左心室腔整體延遲顯影、主動脈延遲顯影、下腔大靜脈擴張、肝臟腫大、顯影劑逆流進入上腔大靜脈、下腔大靜脈與肝靜脈等等現象可以幫助診斷鬱血性心臟衰竭。這些不尋常的顯影現象被認為是血流速度減慢以及心搏輸出降低導致靜脈血壓被動性升高的結果。

（童綜合醫誌 2009; 3: 103-106）

關鍵詞：電腦斷層、左心室腔延遲顯影、顯影劑逆流進入肝靜脈、鬱血性心臟衰竭

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童綜合醫學雜誌投稿相關規則

本雜誌刊載與醫學有關之論述，包括原著論文、臨床病理討論、病例報告等論述及特別約稿之綜論（review article）、special article、Editorial（編著的話）等。惠稿請送 43503 台中縣梧棲鎮中棲路一段 699 號童綜合醫學雜誌編審委員會。

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貳、寫作原則

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2. 病例報告按下列順序撰寫：摘要、前言、病例、討論、參考文獻、附表、圖片說明、附圖、照片。
3. 病例報告，每篇以五頁以內爲限（即約 9,000 字），依題目、所屬機構、作者姓名（作者以 5 人爲限）、病例之病史經過及重要之診療資料、主要之臨床問題、討論或分析、結論、推薦讀物等順序繕寫。凡病患顏面部位之相片必須遮去眼睛部位，表示尊重隱私。診療資料或臨床經過之圖表，原則上均限六個月以內。
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參、投稿須知

一、稿件須符合「生物醫學雜誌投稿之統一規定」[1]，請以電腦隔行 double space 書寫並編頁碼。

二、第一頁爲標題頁，須列出中文及英文之論文題目、簡題（running title）、中英文作者姓名、所屬機構及單位之中英文稱號（分屬不同單位，請以阿拉伯數字標出作者與單位）、聯絡人姓名、電話及中英文通訊錄。

三、第二、三頁爲中文及英文之摘要及關鍵詞 (請提供 3 至 5 個關鍵詞或簡短片語)，中英文摘要須完全相同，英文摘要不超過 250 字，中文摘要不超過 500 字，摘要分段撰寫，依序爲背景及目的（Background and purpose）、方法（Methods）、結果（Results）及討論（Discussion）。

四、請附三份原稿（一份原稿和兩份複印稿，但圖片應使用原圖），包括附表、附圖及照片。圖表應專業製作，一張紙僅一個附圖或附表，依引用順序以阿拉伯數字標出排列。附表須有標題及說明。照片須 5 × 7 吋光面黑白，背面以鉛筆編號，附圖須有簡單說明（Legend），並另頁撰寫。光學或電子顯微鏡照片，請註明擴大倍率或比例。

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一、雜誌名稱之簡稱須按照 Index Medicus 型式，作者人數小於 6 位時，詳列所有作者姓名，超過 6 位時，只須列出前 6 位，其它以「等」（et al）代替。

例：Bhasin S, Storer TW, Berman N, Callegari C, Clecenger B, Phillips J, et all. The effects of supraphysiologic doses of testosterone on muscle size and strength in normal men. N Engl J Med 1996; 335: 1-7.

二、本文內引用時，若兩名以下作者請列出姓氏。兩名以上則列出第一名之姓氏，其他以「等」（et al）代替，並以阿拉伯數字方括弧表示於引用之後。

例：One of the first well documented reports of ECH poisoning with fatality in young children was reported by Miller et al. in 1970[2].

例：Boulet 等人 [3] 報告氣喘患者接受衛教後的知識改變量不受個人因素影響。

三、參考範例

A. 期刊：[作者姓名：題目。雜誌簡稱　年代；卷數（期數）：起迄頁數]

1. 許吟姿、楊光道、張恆鴻：結締組織疾病併發間質性肺病變患者 99mTc-DTPA 肺廓清率之臨床研究。內科學誌 1992;3:79-83.
2. Yang KTA, Chen HD: A semi-automated method for edge detection in the evaluation of left ventricular function using ECG-gated single-photon emission tomography. Eur J Nucl Med 1994; 21: 1206-11.

B. 單行本：[作者姓名：書名，版數（卷數）。發行地；出版公司，年代：引用部份頁數]。

1. 楊志良：生物統計學新論，一版。台北；巨流圖書公司，1984：33-8.
2. Plum F, Posner JB: Diagnosis of Stupor and Coma. 3rd ed. Philadelphia: Davis, 1980:132-3.

C. 多重作者之單行本：[有關文章作者姓名：書名，版數（卷數）。發行地；出版公司，年代：引用部份頁數]。

1. 蔣欣欣：護理與健康，編輯：顧乃平：護理專業導論，一版。台北；匯華出版公司，1991：83-121。
2. Levinsky NG: Fluid and electrolytes. In: Thorn GW, Adams RD, Braunwald E, lsselbacher K, Petersdprf RG eds. Harrison's Principles of Internal Medicine, 8th ed. New York: Mcgraw-Hill, 1977:364-75.

伍、著作權

若著作人投稿於本刊經收錄後，版權即歸本院所有，除本院同意外不得轉載。若爲摘譯、譯稿或改寫稿，需附原作者之正本同意書，並附原文影本一份；來稿如涉及版權，概由作者自負文責。

童綜合醫學雜誌

綜　論

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