ISSN 2071-3592

童綜合醫學雜誌

Tungs' Medical Journal

Volume 7 Number 1 June 2013

TUNGS' MEDICAL JOURNAL

Editorial Office:

The Tungs' Medical Journal, Tungs' Taichung MetroHarbor Hospital.

No.699, Sec. 8, Taiwan Blvd., Wuqi Dist., Taichung City 43503, Taiwan (R.O.C.)

E-Mail: Tungs_Journal@ms.sltung.com.tw

Tel.: 886-4-26581919 ext. 58047 Fax: 886-4-26582193

Printing Company:

Great C Printing Co.

Tel: 886-2-2302-3939 Fax: 886-2-2302-2036

Vol. 7 No. 1 January-June 2013

Tungs' Medical Journal

CONTENTS IN BRIEF

Review Article

Oxidative Stress in Diabetic Nephropathy

Huei-Jane Lee[1,*] Wen-Chin Lee[2,3]

[1] *Institute of Biochemistry and Biotechnology, Medical College, Chung Shan Medical University, Taichung, Taiwan;*
[2] *Division of Nephrology, Department of Internal Medicine, Show Chwan Memorial Hospital, Changhua, Taiwan;*
[3] *General Education Center, Central Taiwan University of Science and Technology, Taichung, Taiwan.*

Received: June. 6, 2013; Accepted: June. 6, 2013

Abstract

Diabetic nephropathy (DN) is one of the complications of diabetes. The presence of high serum glucose concentrations can cause an irreversible progression of renal dysfunction, including glomerulosclerosis, arteriolar hyalinosis, and tubulointerstitial fibrosis. Oxidative stress has been linked to the pathogenesis of DN. The major sources of reactive oxygen species (ROS) in kidney are induced by the polyl pathway, nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, protein kinase C (PKC), and advanced glycation end products (AGEs). A huge increase in ROS can cause protein denaturation and cell death by changing protein sensors or intracellular signaling pathways. In addition to the traditional clinical strategies of controlling glucose and dietary protein, providing antihypertensive treatment, and reducing hyperlipidemia, there are several new approaches to the treatment of diabetic nephropathy that are based on decreasing ROS. Here we review recent research describing the relationship between oxidative stress and DN, and the application of antioxidants to the treatment of DN.

Key words: oxidative stress, diabetic nephropathy, antioxidants

Introduction

Diabetic nephropathy (DN), the major micorvascular complication of diabetes, is the leading cause of end-stage renal failure in several individuals worldwide[1]. In general, about 20–40% of patients with type 1 or type 2 diabetes eventually develop significant DN[2]. The pathogenesis and clinical course of DN can be observed through structural and hemodynamic changes. The earliest change is an increase in the glomerular filtration rate (GFR), also called the "hyperfiltration" stage. Detectable glomerular lesions appear with a normal albumin excretion rate. The next change is the development of microalbuminuria. As microalbuminuria persists, there are changes in glomerular structure, such as mesangial expansion, basement membrane thickening, and an increase in permeability. This stage is referred to as "incipient nephropathy." Diabetic subjects with persistent microalbuminuria are at increased risk for "overt diabetic nephropathy." At this stage, symptoms progress to prominent proteinuria, hypertension, and renal insufficiency. The pathological findings at this stage include glomerular basement membrane (GBM) thickening, mesangial expansion, diffuse and/or nodular glomerulosclerosis, afferent and efferent arteriolar hyalinosis, and tubulointerstitial fibrosis[3]. After several years of persistent proteinuria, progression to end-stage renal disease will occur[4]. Advanced diabetic glomerulopathy is commonly characterized by diffuse glomerulosclerosis and may sometimes exhibit a distinctive morphological appearance, namely the nodular form of glomerulosclerosis, as first described by Kimmelstiel and Wilson in 1936[5,6].

The current strategies to treat diabetic nephropathy include intensive glycemic control, antihypertensive treatment with a particular focus on the interruption of the renin–angiotensin–aldosterone

*Correspondence to: Huei-Jane Lee, Department of Biochemistry, Institute of Biochemistry and Biotechnology, Medical College, Chung Shan Medical University, No. 110, Sec. 1, Jianguo N. Rd., Taichung, 40201, Taiwan (R.O.C.)

system (RAS), restriction of dietary protein, and treatment of hyperlipidemia. There are several new approaches to the treatment of diabetic nephropathy based on an ever-growing mechanistic understanding of the causes of diabetic nephropathy by specific pathogenic processes. These agents include pharmacologic inhibitors of advanced glycation end product (AGE) formation, protein kinase C (PKC), oxidative stress (Sirt 1, Nox 4), and transforming growth factor β (TGF-β)[7-10].

In addition to the clinical treatments for DN, there are also some natural products possessing the potential for alternative applications, such as Hibiscus sabdariffa L. and luteolin. The basis for using these products in ameliorating the progression of DN can be concluded to be their antioxidative abilities. Recently, researchers have found that apocynin or cilostazol also improved oxidative status and further decelerated the advance toward DN. Oxidation and DN are intimately related. According to previous studies, complementing therapies with antioxidants is a good strategy. On the basis of these studies, the relative mechanisms should be reviewed to associate oxidative stress and DN for future applications.

Animal models of diabetes mellitus

Type 1 diabetes mellitus is typically the result of an immune-mediated destruction of the pancreatic β cells. Type 2 diabetes mellitus is characterized by insulin resistance and insulin secretion impairment. As the population of type 1 diabetics lack insulin, the resulting complications are directly related to their high serum glucose concentration, in combination with insulin resistance. To clarify the relative mechanisms, animal models have been used extensively in the study of diabetes. The currently available animal models of type 1 and type 2 diabetes are shown in Table 1[11].

Table 1. Animal models of type 1 and type 2 diabetes mellitus

Type 1	BB (Bio breeding) rat
	Chinese hamster
	Celebes black ape
	Keeshond dog
	LETL (Long Evans Tokushima lean) rat
	New Zealand white rabbit
	NOD (non-obese diabetic) mouse
	Streptozotocin-induced rats
Type 2	CBA/Ca mouse
	db/db mouse
	Diabetic Torri rat
	GK (Goto Kakizaki) rat
	Israeli sand rat
	KK mouse
	New Zealand obese mouse
	NSY (Nagoya–Shibata–Yasuda) mouse
	Ob/Ob mouse
	OLETF (Otsuka Long-Evans Tokushima fatty) rat
	Zucker rat

High glucose causes oxidative stress in diabetic nephropathy

Oxidative stress has emerged as a critical player in the development of DN. Long-term exposure to high glucose concentrations is accompanied by increasing concentrations of reactive oxygen species (ROS) in the kidney. Under this circumstance, a number of redox-sensitive mechanisms would advance some of the pathologies of DN, such as glomerular and tubular hypertrophy, mesangial cell injury, extracellular matrix accumulation, and thickening of glomerular/tubular basement membranes. Following podocyte dysfunction, it would lead to proteinuria, glomerulosclerosis, and tubulointerstitial fibrosis[1,2,12-16].

During the progression of DN, in addition to renin–angiotensin II system-dependent hemodynamic changes and renal hypoxia, there are four major biochemical pathways involved in generating ROS, which are considered to lead to the development of diabetic complications associated with hyperglycemia[17,18]. In the first pathway, the polyol pathway, glucose is converted to sorbitol and then metabolized to fructose. The formation of AGEs and ROS also occurs via this pathway. In the second pathway, the hexosamine pathway, fructose-6-phosphate is converted to glucosamine intermediates and the production of ROS is subsequently increased. In the third pathway, the protein kinase C (PKC) pathway, glucose is converted to glyceraldehyde-3-phosphate, which leads to the formation of diacylglycerol (DAG) [19]. The elevated level of intracellular DAG activates PKC, which then activates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase to induce the formation of ROS. In the fourth and final pathway, the formation of AGEs, interaction of AGEs with the receptors of advanced glycation end-products

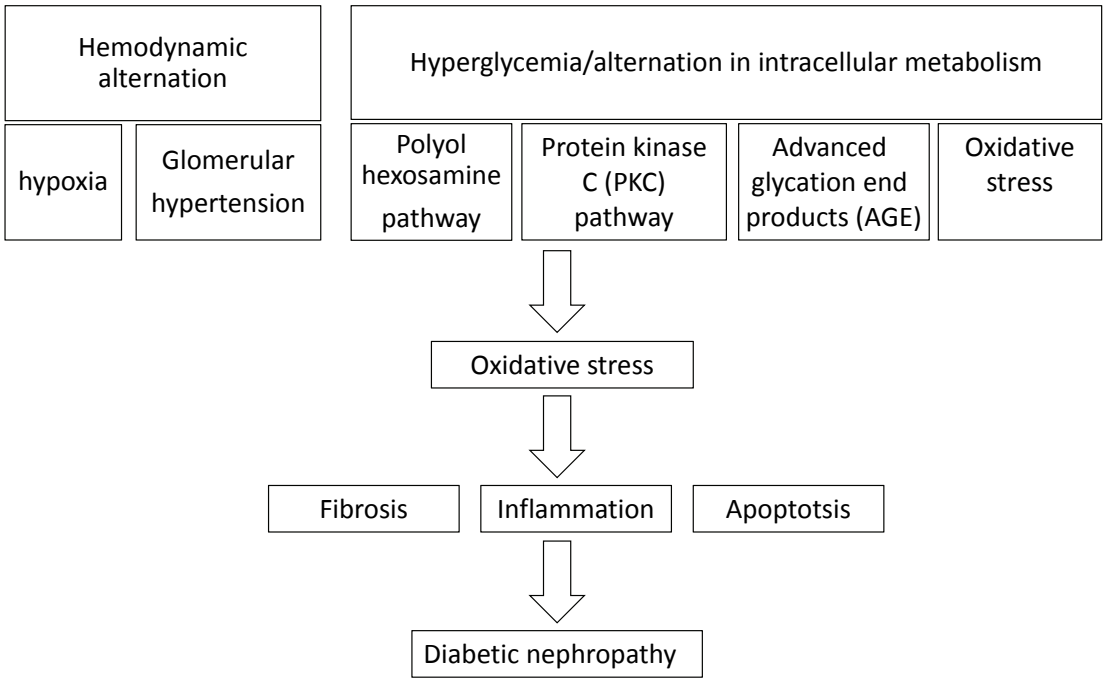


Fig. 1 The relationship between oxidative stress and diabetic nephropathy Oxidative stress can be induced by hyperglycemia-mediated intracellular metabolic alterations, renin-angiotensin II system-dependent hemodynamic changes, and renal hypoxia. This increase in reactive oxygen species (ROS) is highly relevant to the development of diabetic nephropathy.

(RAGEs) results in ROS activation[12,14,20-30] (Fig. 1).

Excess free radicals induce damage to cellular proteins, membrane lipids, and nucleic acids and then cause cell death[31,32]. Increased ROS can also cause vascular endothelial abnormalities by reacting directly with nitric oxide (NO) to produce cytotoxic peroxynitrite, resulting in increased reactivity to vasoconstrictors, and modification of extracellular matrix proteins[33]. ROS also damage endothelial cells indirectly by stimulating the expression of various genes involved in inflammatory pathways[34]. Previous studies have shown that high glucose concentrations induce ROS and upregulate TGF-β1 and extracellular matrix (ECM) expression in glomerular mesangial cells[16,35]. Several lines of evidence prove that antioxidants can effectively inhibit high glucose-induced TGF-β1 and fibronectin upregulation[36]. Ha et al. reported that ROS mediate high glucose-induced activation of NF-κB- and NF-κB-dependent monocyte chemoattractant protein (MCP)-1 expression. NF-κB, a nuclear transcription factor, can initiate the transcription of genes associated with inflammatory responses[37]. It is induced by various cell stress-associated stimuli, including growth factors, vasoactive agents, cytokines, and oxidative stress[38]. AGEs induced by hyperglycemia stimulate NF-κB activation, which sustains the activation of NF-κB in diabetes[39]. Increased steady-state mRNA levels of inflammatory genes have been shown to be associated with interstitial fibrosis and progressive human diabetic nephropathy[38].

TGF-β is important in the development of renal hypertrophy and the accumulation of extracellular matrix (ECM) components in diabetes mellitus[40]. An increase in the expression of TGF-β was observed in diabetic nephropathy of experimental animals and in humans[41-44]. Treatment with anti-TGF-β antibody has been documented to attenuate the effects of high glucose-induced cellular hypertrophy in vitro and in streptozotocin-induced diabetic mice[45-47]. TGF-β is also the key regulator of ECM remodeling in the mesangium, causing mesangial expansion and inducing the process of epithelial–mesenchymal transition (EMT), which causes tubulointerstitial fibrosis[48,49]. With the accumulation of ECM and the persistence of tubulointerstitial fibrosis, renal function progresses to end-stage renal disease (ESRD).

Sirt1 is a NAD^+-dependent deacetylase. It

functions as an intracellular energy sensor, monitoring the concentration of NAD^+ and controlling the metabolic changes induced by caloric restriction and starvation through its deacetylase activity toward many targets, including histones and nuclear transcriptional factors. Disturbance of Sirt1 activation would cause the onset of obesity-associated diseases, such as diabetes[8]. Sirt1 is found in mesangial, epithelial, and endothelial cells, except in those of the glomerulus. Sirt1 expression is decreased in streptozotocin-induced and type 2 diabetic rats[50,51]. It is found to inhibit the activation of NFkB via deacetylation of an NFkB subunit (p65) to exert its anti-inflammatory effects[52,53]. Sirt1 effects occur not only in type 1 diabetes but are also observed in type 2 diabetes with DN. Kitada et al. showed that, in Wistar fatty rats with diabetes, severe renal lesions with inflammatory cell infiltration appeared in the kidneys, accompanying a significant reduction of Sirt1 expression and acetylation of p65. Caloric restriction, in particular, induced the deacetylation of p65 and led to a reduction in inflammation. This implies that Sirt1 activation is involved in the caloric restriction-mediated reduction in inflammation in DN. Sirt1 was also found to induce the expression of catalase, an anti-oxidative enzyme, by activating the transcriptional factor Foxo3a. This inhibits apoptosis resulting from oxidative stress[54]. It is an alternative concept that Sirt1 activation would be a new strategy for treating or preventing the progression of DN.

Nox4, which belongs to the NADPH oxidase (Nox) family, is abundant in renal systems, including renal tubules, renal fibroblasts, glomerular mesangial cells, and podocytes[28,55-62]. Nox4 levels increase in the mesangium, glomeruli, and tubular cells in DN. High glucose concentrations induce the expression of Nox4 protein and the generation of ROS. Gorin et al. pointed out that Nox4-dependent ROS generation mediates glomerular hypertrophy and mesangial matrix accumulation[28]. Inhibiting Nox4 expression by administration of anti-sense oligonucleotides for Nox4 reduced not only glomerular enlargement and fibronectin accumulation but also the generation of ROS in DN of type 1 diabetic rats. Previous studies have shown that Nox4 is involved in many cellular signaling pathways, such as IGF-1, TGF-β, and angiotensin II pathways. Under hyperglycemic conditions, Nox4 can engage in cross-talk with additional cellular signaling pathways, including PKC, AMPK, and PLA2 pathways. This results in the development of increasing extracellular matrix, hypertrophy, and TGF-β and also causes podocyte dysfunction and apoptosis.

Application of antioxidants in DN

ROS play an important role in the pathogenesis of DN[63]. To prevent the development and progression of diabetic nephropathy, it would be effective to decrease overproduction of ROS and/or to increase the removal of preformed ROS[64]. Some natural products have been shown to possess the ability to decelerate diabetic nephropathy by reducing oxidative stress. Owing to the presence of polyphenolic acids, flavonoids, protocatechuic acid, and anthocyanins, Hibiscus sabdariffa L. extract (HSE) has been shown to have the antioxidative potential to inhibit the development of atherosclerosis in cholesterol-fed rabbits, LDL oxidation, and ox-LDL-mediated macrophage apoptosis[65-68]. Wang et al. demonstrated that HSE is capable of significantly increasing catalase and glutathione activities in DN[69]. In histological examinations, HSE improves the hydropic change of renal proximal convoluted tubules in diabetic rats. HSE was also observed to upregulate Akt/Bad/14-3-3 and NF-κB-mediated transcription. Luteolin is a plant-derived flavonoid having various biological activities, including antiinflammatory[70], antimutagenic, and antitumorigenic properties[71]. It also possesses direct antioxidant activity[72] and may be useful in the treatment of many chronic diseases associated with oxidative stress, such as cardiovascular diseases[73,74], liver diseases[75,76], diabetes[77], and aging[78]. Wang et al. demonstrated that luteolin protects against the development of DN by changing superoxide dismutase (SOD) activity, malondialdehyde (MDA) content, and expression of Heme Oxygenase-1 (HO-1) protein[79].

There is some evidence that exogenous or endogenous antioxidants can also decelerate the progression of DN. Oxidative stress via NADPH oxidase and the vascular endothelial growth factor (VEGF) pathway are documented to play important roles in the development of DN. Nam et al. demonstrated the effects of apocynin, a NADPH oxidase inhibitor, on DN[30]. They showed that apocynin cannot significantly decrease serum glucose levels but is capable of reducing urinary protein and albumin excretion. Apocynin treatment improved

glomerular and mesangial expansion. Apocynin also decreased glomerular VEGF expression and reduced the 24-h urinary concentrations of 8-OHdG and MDA, which are markers of oxidative damage to DNA and polyunsaturated lipids, respectively. Lee et al. also demonstrated that the antioxidant taurine prevented glomerular hypertrophy, mesangial expansion, and proteinuria in diabetic rats[80]. Additionally, Craven et al. revealed that diabetic mice transgenic for Cu/Zn SOD had significantly lower urinary albumin excretion, glomerular hypertrophy, and glomerular expression of TGF-β1 and collagen IV protein[81]. Hamada et al. demonstrated that overexpression of a small antioxidant, thioredoxin 1, effectively inhibited production of 8-OHdG in the kidney, albuminuria, mesangial expansion, and tubular injury in diabetic mice[82]. Du et al. found that overexpression of MnSOD in bovine aortic endothelial cells prevented high glucose-induced activation of the PKC, NK-κB, hexosamine, and AGE pathways[83]. Brezniceanu et al. demonstrated that renal catalase overexpression in db/db mice attenuated ROS generation, angiotensinogen production, proapoptotic gene expression, and apoptosis in the kidneys of diabetic mice in vivo[84].

Many of the current standard therapeutic approaches may also ameliorate oxidative stress as pleiotropic effects[14], such as angiotensin-2 converting enzyme (ACE) inhibitors[85], angiotensin-2 receptor blockers (ARB)[86], and aldosterone blockers (spironolactone)[87]. They activate eNOS to increase the bioavailability of nitric oxide, to inhibit synthesis of angiotensin 2 and TGF-β, and to decelerate or prevent tubulointerstitial fibrosis in diabetic nephropathy, accompanied by control of systemic and intrarenal blood pressure. Cilostazol is a specific inhibitor of phosphodiesterase 3 (PDE 3)[88-90]. It has been shown that cilostazol significantly decreases ROS activity in the kidneys of diabetic rats; improves the urine albumin/creatinine ratio; and reduces the diabetes-induced increase in glomerular size, TGF-β, and NF-κB in early DN[91]. Lipid-lowering agents such as statins, which can inhibit HMG-CoA reductase, have been demonstrated to activate eNOS, maintain the glomerular filtration rate and renal cortical blood flow, and ameliorate glomerular lesions[92,93]. Benfotiamine was used in the treatment of DN to decrease hyperfiltration and proteinuria and has also been demonstrated to reduce ROS formation[94]. Potential therapies using these ideal antioxidants would influence the pathways of ROS generation to decelerate the progression of DN.

Conclusion

High serum glucose concentrations result in the progression of DN in type 1 diabetes, partly because of increasing oxidative stress. In addition to the standard glycemic control and antihypertensive treatments, there are antioxidants, such as clinical drugs or natural products, possessing the ability to decelerate the development of DN. The auxiliary use of these antioxidants may be a complementary and alternative medical concept for controlling DN.

References

1. Hakim FA, Pflueger A. Role of oxidative stress in diabetic kidney disease. Med Sci Monit 2010;16:RA37-48.
2. Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res 2010;107:1058-70.
3. Cooper ME, Gilbert RE. Pathogenesis, prevention, and treatment of diabetic nephropathy, In Comprehensive clinical nephrology 2nd edition (Johnson RJ, and Feehally J, eds) 2003; pp 439-50, Mosby, Edinburgh.
4. Caramori ML, Mauer M. Diabetic nephropathy. in Primer on kidney diseases, 3rd edition (Greenberg A, Cheung AK, Coffman TM, Falk RJ, and Jennette JC eds) 2001; pp 212-8, National Kidney Fundation, Florida.
5. Kimmelstiel P, Wilson C. Intercapillary lesions in glomeruli of the kidney. Am J Pathol 1936;12:83-97.
6. Kern WF, Laszik ZG, Nadasdy T, Silva FG, Bane BL, Pitha JV. The kidney in metabolic disorder-- diabetes mellitus, hyperuricemia, oxalosis, nephrocalcinosis, and nephrolithiasis. in Atlas of renal pathology (Kern WF, Laszik ZG, Nadasdy T, Silva FG, Bane BL, and Pitha JV, eds) 1999; pp 97-112, W.B. Saunders company, Philadelphia.
7. Kitada M, Kume S, Takeda-Watanabe A, Kanasaki K, Koya D. Sirtuins and renal diseases: relationship with aging and diabetic nephropathy. Clinic Sci 2013;124: 153-64.
8. Kume S, Kitada M, Kanasaki K, Maegawa H, Koya D. Anti-aging molecule, sirt1: a novel therapeutic target for diabetic nephropathy. Arch Pharm Res 2013;36:230-6.
9. Gorin Y, and Block K. Nox4 and diabetic nephropathy: with a friend like this, who needs enemies? Free Radic Biol Med 2013;61:130-42.
10. Williams ME, Stanton RC. Management of diabetic kidney disease, in Joslin's Diabetes Mellitus (Kahn CR, Weir GC, King GL, Jacobson AM, Moses AC, and Smith RJ, eds) 2005; pp 925-49, Lippincott Williams & Wilkins, Philadelphia.
11. Rees DA, Alcolado JC. Animal models of diabetes mellitus. Diabet Med 2005;22: 359-70.
12. Forbes JM, Coughlan MT, Cooper ME. Oxidative stress as a major culprit in kidney disease in diabetes. Diabetes 2008; 57:1446-54.
13. Kashihara N, Haruna Y, Kondeti VK, Kanwar YS. Oxidative stress in diabetic nephropathy. Curr Med Chem 2010;17: 4256-69.

14. Singh DK, Winocour P, Farrington K. Oxidative stress in early diabetic nephropathy: fueling the fire. Nat Rev Endocrinol 2011;7:176-84.
15. Stanton RC. Oxidative stress and diabetic kidney disease. Curr Diabetes Rep 2011; 11:330-6.
16. Susztak K, Raff AC, Schiffer M, Bottinger EP. Glucose-induced reactive oxygen species cause apoptosis of podocytes and podocyte depletion at the onset of diabetic nephropathy. Diabetes 2006;55:225-33.
17. Campbell KN, Raij L, Mundel P. Role of angiotensin II in the development of nephropathy and podocytopathy of diabetes. Curr Diabetes Rev 2011;7:3-7.
18. Rincon-Choles H, Kasinath BS, Gorin Y, Abboud HE. Angiotensin II and growth factors in the pathogenesis of diabetic nephropathy. Kidney Int Suppl 2002;82:S8-11.
19. Thallas-Bonke V, Thorpe SR, Coughlan MT, Fukami K, Yap FY, Sourris KC, et al. Inhibition of NADPH oxidase prevents advanced glycation end product-mediated damage in diabetic nephropathy through a protein kinase C-alpha-dependent pathway. Diabetes 2008;57:460-9.
20. Stirban A, Rosen P, Tschoepe D. Complications of type 1 diabetes: new molecular findings. Mt Sinai J Med 2008;75: 328-51.
21. Shah IM, Mackay SP, McKay GA. Therapeutic strategies in the treatment of diabetic nephropathy- a translational medicine approach. Curr Med Chem 2009;16:997-1016.
22. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes 2005;54:1615-25.
23. Kanwar YS, Wada J, Sun L, Xie P, Wallner EI, Chen S, et al. Diabetic nephropath: mechanisms of renal disease progression. Exp Biol Med (Maywood) 2008;233:4-11.
24. Winiarska K, Malinska D, Szymanski K, Dudziak M, Bryla J. Lipoic acid ameliorates oxidative stress and renal injury in alloxan diabetic rabbits. Biochimie 2008;90: 450-9.
25. Yi X, Nickeleit V, James LR, Maeda N. alpha-Lipoic acid protects diabetic apolipoprotein E-deficient mice from nephropathy. J Diabetes Compli 2011;25:193-201.
26. Zheng S, Carlson EC, Yang L, Kralik PM, Huang Y, Epstein PN. Podocyte-specific overexpression of the antioxidant metallothionein reduces diabetic nephropathy. J Am Soc Nephrol 2008;19: 2077-85.
27. Asaba K, Tojo A, Onozato ML, Goto A, Quinn MT, Fujita T, et al. Effects of NADPH oxidase inhibitor in diabetic nephropathy. Kidney Int 2005;67:1890-8.
28. Gorin Y, Block K, Hernandez J, Bhandari B, Wagner B, Barnes JL, Abboud HE. Nox4 NAD(P)H oxidase mediates hypertrophy and fibronectin expression in the diabetic kidney. J Biol Chem 2005;280: 39616-26.
29. Hwang I, Lee J, Huh JY, Park J, Lee HB, Ho YS, et al. Catalase deficiency accelerates diabetic renal injury through peroxisomal dysfunction. Diabetes 2012;61: 728-38.
30. Nam SM, Lee MY, Koh JH, Park JH, Shin JY, Shin YG, et al. Effects of NADPH oxidase inhibitor on diabetic nephropathy in OLETF rats: the role of reducing oxidative stress in its protective property. Diabetes Res Clin Pract 2009;83: 176-82.
31. Maritim AC, Sanders RA, Watkins JB 3rd. Diabetes, oxidative stress, and antioxidants: A review. J Biochem Mol Toxicol 2003;17: 24-38.
32. Djordjevic VB. Free radicals in cell biology. Int Rev Cytol 2004;237:57-89.
33. Schnackenberg CG. Physiological and pathophysiological roles of oxygen radicals in the renal microvasculature. Am J Physiol Regul Integr Comp Physiol 2002;282: R335-R42.
34. Baldwin AS Jr. The NF-κB and IκB proteins: new discoveries and insights. Annu Rev Immunol 1996;14:649-83.
35. Lee HB, Yu MR, Yang Y, Jiang Z, Ha H. Reactive oxygen species-regulated signaling pathways in diabetic nephropathy. J Am Soc Nephrol 2003;14: S241-5.
36. Ha H, Lee SH, Kim KH. Effects of rebamipide in a model of experimental diabetes and on the synthesis of transforming growth factor-β and fibronectin, and lipid peroxidation induced by high glucose in cultured mesangial cells. J Pharmacol Exp Ther 1997;281: 1457-62.
37. Ha H, Yu MR, Choi YJ, Kitamura M, Lee HB. Role of high glucose-induced nuclear factor-κB activation in monocyte chemoattractant protein-I expression by mesangial cells. J Am Soc Nephrol 2002;13:894-902.
38. Kuhad A, Chopra K. Attenuation of diabetic nephropathy by tocotrienol: involvement of NF-κB signaling pathway. Life Sci 2009;84:296-301.
39. Gao L, Wang F, Wang B, Gong B, Zhang J, Zhang X, et al. Cilostazol protects diabetic rats from vascular inflammation via nuclear factor-κB-dependent down-regulation of vascular cell adhesion molecule-1 expression. J Pharmacol Exp Ther 2006;318:53-8.
40. Wolf G, and Ziyadeh FN. Molecular mechanisms of diabetic renal hypertrophy. Kidney Int 1999;56:393-405.
41. Park IS, Kiyomoto H, Abboud SL, Abboud HE. Expression of transforming growth factor-β and type IV collagen in early streptozotocin- induced diabetes. Diabetes 1997;46: 473-80.
42. Yamamoto T, Nakamura T, Noble NA, Ruoslahti E, Border WA. Expression of transforming growth factor-β is elevated in human and experimental diabetic nephropathy. Proc Natl Acad Sci USA 1993;90:1814-8.
43. Sharma K, Ziyadeh FN, Alzahabi B, McGowan TA, Kapoor S, Kurnik BR, et al. Increased renal production of transforming growth factor-beta 1 in patients with type II diabetes. Diabetes 1997;46:854-9.
44. Shankland SJ, Scholey JW, Ly H, Thai K. Expression of transforming growth factor-β1 during diabetic renal hypertrophy. Kidney Int 1994;46:430-42.
45. Wolf G, Sharma K, Chen Y, Ericksen M, Ziyadeh FN. High glucose- induced proliferation in mesangial cells is reversed by autocrine TGF-β. Kidney Int 1992;42: 647-656.
46. Ziyadeh FN, Sharma K, Ericksen M, Wolf G. Stimulation of collagen gene expression and protein synthesis in murine mesangial cells by high glucose is mediated by activation of transforming growth factor-β. J Clin Invest 1994;93: 536-42.
47. Sharma K, Jin Y, Guo J, Ziyadeh FN. Neutralization of TGF-beta by anti-TGF-beta antibody attenuates kidney hypertrophy and the enhanced extracellular matrix gene expression in STZ-induced diabetic mice. Diabetes 1996; 45:522-30.
48. Ziyadeh FN, Hoffman BB, Han DC, Iglesias-De La Cruz MC, Hong SW, Isono M, et al. Long-term prevention of renal insufficiency, excess matrix gene expression, and glomerular mesangial matrix expansion by treatment with monoclonal anti-transforming growth factor-beta antibody in db/db diabetic mice. Proc Natl Acad Sci USA 2000;97:8015-20.
49. Oldfield MD, Bach LA, Forbes JM, Nikolic-Paterson D, McRobert A, Thallas V, et al. Advanced glycation end products cause epithelial-myofibroblast transdifferentiation via the receptor for advanced glycation end products (RAGE). J Clin Invest 2001;108: 1853-63.

50. Kume S, Uzu T, Horiike K, Chin-Kanasaki M, Isshiki K, Araki S, et al. Calorie restriction enhances cell adaptation to hypoxia through Sirt1-dependent mitochondrial autophagy in mouse aged kidney. J Clin Invest 2010;120: 1043–55.
51. Tikoo K, Singh K, Kabra D, Sharma V, Gaikwad A. Change in histone H3 phosphorylation, MAP kinase p38, SIR 2 and p53 expression by resveratrol in preventing streptozotocin induced type I diabetic nephropathy. Free Radic Res 2008; 42:397-404.
52. Yeung F, Hoberg JE, Ramsey CS, Keller MD, Jones DR, Frye RA, et al. Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. EMBO J 2004;23:2369-80.
53. Yoshizaki T, Schenk S, Imamura T, Babendure JL, Sonoda N, Bae EJ, et al. SIRT1 inhibits inflammatory pathways in macrophages and modulates insulin sensitivity. Am J Physiol Endocrinol Meta 2010;298:E419-E28.
54. Hasegawa KS, Wakino K, Yoshioka S, Tatematsu Y, Hara H, Minakuchi K, et al. Kidney-specific overexpression of Sirt1 protects against acute kidney injury by retaining peroxisome function. J Biol Chem 2010;285:13045-56.
55. Gorin Y, Ricono JM, Kim NH, Bhandari B, Choudhury GG, Abboud HE. Nox4 mediates angiotensin II-induced activation of Akt/protein kinase B in mesangial cells. Am J Physiol Renal Physiol 2003;285: F219-29.
56. Gorin Y, Ricono JM, Wagner B, Kim NH, Bhandari B, Choudhury GG, et al. Angiotensin II-induced ERK1/ERK2 activation and protein synthesis are redox-dependent in glomerular mesangial cells. Biochem J 2004;381:231-9.
57. Schreck C, O'Connor PM. NAD(P)H oxidase and renal epithelial ion transport. Am J Physiol Regul Integr Comp Physiol 2011;300:R1023-9.
58. Bondi CD, Manickam N, Lee DY, Block K, Gorin Y, Abboud HE, et al. NAD(P)H oxidase mediates TGF-beta1-induced activation of kidney myofibroblasts. J Am Soc Nephrol 2010;21:93-102.
59. Eid AA, Gorin Y, Fagg BM, Maalouf R, Barnes JL, Block K, et al. Mechanisms of podocyte injury in diabetes: role of cytochrome P450 and NADPH oxidases. Diabetes 2009;58: 1201-11.
60. Geiszt M, Kopp JB, Varnai P, Leto TL. Identification of renox, an NAD(P)H oxidase in kidney. Proc Natl Acad Sci USA 2000;97:8010-4.
61. Shiose A, Kuroda J, Tsuruya K, Hirai M, Hirakata H, Naito S, et al. A novel superoxide-producing NAD(P)H oxidase in kidney. J Biol Chem 2001;276:1417-23.
62. Ambasta RK, Kumar P, Griendling KK, Schmidt HH, Busse R, Brandes RP. Direct interaction of the novel Nox proteins with p22phox is required for the formation of a functionally active NADPH oxidase. J Biol Chem 2004;279: 45935-41.
63. Rösen P, Nawroth PP, King G, Möller W, Tritschler HJ, Packer L. The role of oxidative stress in the onset and progression of diabetes and its complications: a summary of a Congress Series sponsored by UNESCO-MCBN, the American Diabetes Association and the German Diabetes Society. Diabetes Metab Res Rev 2001;17:189-212.
64. Ha H, Hwang IA, Park JH, Lee HB. Role of reactive oxygen species in the pathogenesis of diabetic nephropathy. Diabetes Res Clin Pract 2008; 82(Suppl 1): S42-5.
65. Chen CC, Hsu JD, Wang SF, Chiang HC, Yang MY, Kao ES, et al. Hibiscus sabdariffa Extract inhibits the development of atherosclerosis in cholesterol-fed rabbits. J Agric Food Chem 2003;51:5472-7.
66. Chang YC, Huang KX, Huang AC, Ho YC, Wang CJ. *Hibiscus* anthocyanins- rich extract inhibited LDL oxidation and oxLDL-mediated macrophages apoptosis. Food Chem Toxicol 2006;44:1015-23.
67. Faraji M, Tarkhani A. The effect of sour tea (*Hibiscus sabdariffa*) on essential hypertension. J Ethnopharmacol 1999;65:231-6.
68. Tseng TH, Kao TW, Chu CY, Chou FP, Lin WL, Wang CJ. Induction of apoptosis *Hibiscus* protocatechuic acid in human leukemia cells via reduction of retinoblastoma (RB) phosphorylation and Bcl-2 expression. Biochem Pharmacol 2000;60:307-15.
69. Wang SC, Lee SF, Wang CJ, Lee CH, Lee WC, Lee HJ. Aqueous Extract from Hibiscus sabdariffa Linnaeus Ameliorate Diabetic Nephropathy via Regulating Oxidative Status and Akt/Bad/14-3-3{gamma} in an Experimental Animal Model. Evid Based Complement Alternat Med 2011;doi:10.1093/ecam/nep181.
70. Jang S, Kelley KW, Johnson RW. Luteolin reduces IL-6 production in microglia by inhibiting JNK phosphorylation and activation of AP-1. Proc Natl Acad Sci USA 2008;105: 7534-9.
71. Ross JA, Kasum CM. Dietary flavonoids: bioavailability, metabolic effects, and safety. Annu Rev Nutr 2002;22: 19-34.
72. López-Lázaro M. Distribution and biological activities of the flavonoid luteolin Mini Rev Med Chem 2009; 9:31-59.
73. McCord JM. Oxygen-derived free radicals in postischemic tissue injury. N Engl J Med 1985;312:159-63.
74. Jeroudi MO, Hartley CJ, Bolli R. Myocardial reperfusion injury: role of oxygen radicals and potential therapy with antioxidants. Am J Cardiol 1994; 73: 2B-7B.
75. Comporti M. Lipid peroxidation and cellular damage in toxic liver injury. Lab Invest 1985; 53:599-623.
76. Poli G, Albano E, Dianzani MU. The role of lipid peroxidation in liver damage. Chem Phys Lipids 1987;45:117-42.
77. Oberley LW. Free radicals and diabetes. Free Radic Biol Med 1988;5:113-24.
78. Harman D. The aging process. Proc Natl Acad Sci USA 1981;78:7124-8.
79. Wang GG, Lu XH, Li W, Zhao X, Zhang C. Protective effects of luteolin on diabetic nephropathy in STZ-Induced diabetic rats. Evid Based Complement Alternat Med 2011; doi:10.1155/2011/323171.
80. Lee EA, Seo JY, Jiang Z, Yu MR, Kwon MK, Ha H, et al. Reactive oxygen species mediate high glucose-induced plasminogen activator inhibitor-1 upregulation in mesangial cells and in diabetic kidney. Kidney Int 2005;67:1762-71.
81. Craven PA, Melhem MF, Phillips SL, DeRubertis FR. Overexpression of Cu2+/Zn2+ superoxide dismutase protects against early diabetic glomerular injury in transgenic mice. Diabetes 2001;50:2114-25.
82. Hamada Y, Miyata S, Nii-Kono T, Kitazawa R, Kitazawa S, et al. Overexpression of thioredoxin 1 in transgenic mice suppresses development of diabetic nephropathy. Nephrol Dial Transplant 2007;22:1547-57.
83. Du X, Matsumura T, Edelstein D, Rossetti L, Zsengellé r Z, Szabó C, et al. Inhibition of GAPDH activity by poly(ADP-ribose) polymerase activates three major pathways of hyperglycemic damage in endothelial cells. J Clin Invest 2003;112:1049-57.
84. Brezniceanu ML, Liu F, Wei CC, Tran S, Sachetelli S, Zhang SL, et al. Catalase overexpression attenuates angiotensinogen

expression and apoptosis in diabetic mice. Kidney Int 2007;71:912-23.
85. Kobayashi N, Honda T, Yoshida K, Nakano S, Ohno T, Tsubokou T, et al. Critical role of bradykinin-eNOS and oxidative stress-LOX-1 pathway in cardiovascular remodeling under chronic angiotensin-converting enzyme inhibition. Atherosclerosis 2006;187:92-100.
86. Ogawa S, Mori T, Nako K, Keto T, Takeuchi K, Ito S. Angiotensin II type 1 receptor blockers reduce urinary oxidative stress markers in hypertensive diabetic nephropathy. Hypertension 2006;47:699-705.
87. Takebayashi K, Matsumoto S, Aso Y, Inukai T. Aldosterone blockade attenuates urinary monocyte chemoattractant protein-1 and oxidative stress in patients with type 2 diabetes complicated by diabetic nephropathy. J Clin Endocrinol Metab 2006;91: 2214-7.
88. Matsumoto T, Kobayashi T, Wakabayashi K, Kamata K. Cilostazol improves endothelium-derived hyperpolarizing factor-type relaxation in mesenteric arteries from diabetic rats. Am J Physiol Heart Circ Physiol 2005;289:H1933-H40.
89. Takahashi S, Oida K, Fujiwara R, Maeda H, Hayashi S, Takai H, et al. Effect of cilostazol, a cyclic AMP phosphodiesterase inhibitor, on the proliferation of rat aortic smooth muscle cells in culture. J Cardiovasc Pharmacol 1992;20: 900-6.
90. Ishizaka N, Taguchi J, Kimura Y, Ikari Y, Aizawa T, Togo M, et al. Effects of a single local administration of cilostazol on neointimal formation in balloon-injured rat carotid artery. Atherosclerosis 1999;142:41-6.
91. Lee WC, Chen HC, Wang CY, Lin PY, Ou TT, Chen CC, et al. Cilostazol ameliorates nephropathy in type 1 diabetic rats involving improvement of oxidative stress and regulation of TGF-β1 and NF-κB. Biosci Biotechnol Biochem 2010;74: 1355-61.
92. Usui H, Shikata K, Matsuda M, Okada S, Ogawa D, Yamashita T, et al. HMG-CoA reductase inhibitor ameliorates diabetic nephropathy by its pleiotropic effects in rats. Nephro. Dial Transplant 2003;18:265-72.
93. Endres M, Laufs U. Effects of statins on endothelium and signaling mechanisms. Stroke 2004; 35(Suppl 1):2708-11.
94. Babaei-Jadidi R, Karachalias N, Ahmed N, Battah S, Thornalley PJ. Prevention of incipient diabetic nephropathy by high-dose thiamine and benfotiamine. Diabetes 2003; 52: 2110-20.

糖尿病腎病變之氧化壓力

李彗禎[1,*]　李文欽[2,3]

[1]中山醫學大學　生化暨生物科技研究所
[2]彰化秀傳紀念醫院　腎臟科
[3]中台科技大學　通識教育中心

受文日期：民國 102 年 6 月 6 日；接受刊載：民國 102 年 6 月 6 日

摘要

糖尿病腎病變是糖尿病的併發症之一，長期的高血糖狀態使腎臟出現不可逆的機能逐漸喪失，包括腎絲球硬化、血管變性及腎小管間質纖維化。近期文獻指出"氧化壓力"在糖尿病腎病變的病理化過程有其關聯，在糖尿病人的腎臟中主要的活性氧（reactive oxygen species, ROS）的來源為 polyl 途徑、NADPH 氧化酶、蛋白激酶 C（protein kinase C，PKC）及糖化終產物（advanced glycation end products，AGEs）。大量產生的 ROS 可能經由調節細胞內的蛋白感應器或訊息傳遞路徑而導致糖尿病腎中蛋白變性及細胞死亡而使腎臟病變。基於此類機轉，除了使用傳統的臨床策略來控制糖尿病人的血糖及蛋白攝食量，或使用降血壓或降血脂藥外，最近亦有文獻指出可將降低 ROS 的策略應用於延緩糖尿病腎的發展。在此，我們以文獻回顧的方式針對氧化壓力及糖尿病腎病變的關係做一統整，並介紹抗氧化劑的相關機轉及應用。

關鍵詞：氧化壓力、糖尿病腎病變、抗氧化劑

* 通訊作者：李彗禎教授　中山醫學大學醫學院生化暨生物科技研究所　台中市南區建國北路一段 110 號

Tungs' Medical Journal 7 (2013) 10-17

Original Article

氣喘與非氣喘兒童其尿液 8-hydroxy-2′-deoxyguanosine 濃度之差異

王銘甫[1]　呂克桓[2]　張璧伊[3]　許銘麟[3]　王尊彥[3]　翁瑞宏[3,*]

童綜合醫院兒童醫學部　[1]小兒過敏免疫科
中山醫學大學附設醫院　[2]兒童部
中山醫學大學　[3]公共衛生學系

受文日期：民國 101 年 2 月 1 日；接受刊載：民國 102 年 3 月 29 日

摘要

背景：二手菸（environmental tobacco smoke, ETS）暴露所誘發的氣喘（asthma）可能與反應性氧化產物（reactive oxygen species, ROS）引起的氧化性 DNA 傷害具有相關，而氧化性 DNA 傷害指標 8-hydroxy-2′-deoxyguanosine（8-OHdG）與兒童氣喘之相關性，目前仍未被清楚地探討。
方法：本研究選取 35 名氣喘兒童為病例組，以及 35 名非氣喘兒童為對照。研究對象的個人特徵資料，是經由面對面的問卷訪視所收集；兒童的累積二手菸暴露程度是以抽菸包年來當作指標，此被定義為與兒童共同生活之家戶成員其每天的抽菸包數乘以兒童暴露到二手菸的期間。尿液氧化性 DNA 傷害標記 8-OHdG 是使用酵素連結免疫吸附分析法（enzyme-linked immunosorbent assay, ELISA）來測量。
結果：男孩以及氣喘家族史，在病例組中的比例是明顯高於對照組，氣喘病例相較於對照具有顯著較高的 8-OHdG 濃度（77.4 vs. 17.8 ng/ml creatinine, P = 0.004）。在沒有氣喘的對照組中，男孩相較於女孩（P < 0.001）也具有顯著較高的尿液 8-OHdG 濃度；特別的是，沒有氣喘之對照組孩童每增加一包年的二手菸暴露量，可增加 1.4 ng/mg creatinine 的尿液 8-OHdG 濃度（P < 0.001）。
結論：細胞的氧化性 DNA 傷害可能在兒童氣喘的發生中扮演重要的角色。

關鍵詞：二手菸暴露、氣喘、8-hydroxy-2′-deoxyguanosine

前　言

氣喘（asthma）是種呼吸道疾病，其特徵是受到多重反應物之刺激後，可產生細支氣管痙攣，並導致呼吸困難（dyspnea）而哮喘（wheeze）；因此，在氣喘患者身上經常可以觀察到慢性呼吸道發炎、可逆性氣流阻塞以及支氣管過度反應等現象[1]。先天的遺傳與後天的環境暴露對於氣喘的發生，可能扮演關鍵性的角色；環境中存在著許多過敏性物質，例如內毒素（endotoxin）[2]，容易刺激呼吸道，使免疫 T 細胞、肥大細胞（mast cell）、嗜中性白血球（neutrophils）分泌細胞激素（cytokines）和細胞介質（mediators），而造成血管通透性增加、黏液過度分泌、以及平滑肌收縮且增生（proliferation），因此具有氣喘症狀的人肺功能會降低[3]。目前已知氣喘是受到環境及遺傳因素的共同作用，因此嘗試探討相關環境因子在氣喘兒童體內的特定生物標記之表現，將有助於瞭解氣喘發生之致病機轉。

兒童是氣喘的易好發族群；一項在丹麥所進行的研究指出[4]，氣喘盛行率有兩個年齡高峰，第一高峰是兒童期，主要是受到過敏的影響，而多在成年前即可停止；而第二高峰是 60-70 歲之老年人，主因是氣管加強反應所引起。兒童氣喘在世界各國都有明顯增加的趨勢；而根據 Chen 等人[5] 在 2001 年所發表的研究結果顯示，台灣兒童氣喘盛行率也已高達 8.2%。

由於孩童待在家中之時間（70-90%）相當長，所以

* 通訊作者：翁瑞宏醫師　中山醫學大學　公共衛生學系
40201 台中市南區建國北路一段 110 號

室內空氣污染程度對於兒童健康之影響也受到高度之重視；其中，又以環境菸草煙霧（environmental tobacco smoke, ETS）的暴露與氣喘具有明顯的關連性 [6,7]。香菸在其氣態與焦油態的成分中包含著高濃度的反應性氧化產物（reactive oxygen species, ROS）[8]，包括甲醛（aldehyde）、過氧化物（peroxides）、一氧化氮 vnitric oxide, NO•）、二氧化氮（nitrogen dioxide）、過氧亞硝酸根離子（peroxynitrite, $ONOO^-$）、超氧陰離子（superoxide anion）、氫氧自由基 (hydroxyl radical, OH•）等，這些物質均具有氧化傷害的能力。Pryor 和 Stone[9] 指出每口氣態的香菸中大約包含有 10^{15} 個自由基，並且 NO• 的濃度可高達 500 ppm 以上；Janoff 等人 [10] 也指出每克的香菸焦油中包含有 10^{18} 個自由基。而 ROS 可以造成細胞 DNA 結構改變 [11]，也經常被認爲與發炎有關。氣管上皮細胞（airway epithelial cell, AEC）是位於組織外部區域，容易受到空氣污染物的破壞，而氣管上皮細胞的細胞膜是抵抗侵襲的屏障。從呼吸道表面一直到肺部氣體交換區域都有巨噬細胞的存在，所以巨噬細胞特別容易與吸入的物質作用，而釋放出過氧化氫（hydrogen peroxide, H_2O_2）、OH•、超氧自由基（superoxide, $O_2^{\bullet -}$）以及單重氧（singlet oxygen）。而這些 ROS 會引發細胞激素的增加，造成支氣管上皮細胞發炎，導致氣喘 [11]。另一方面，ROS 與肺部細胞的抗氧化劑如麩氨基硫還原酶（glutathione redusease）、麩氨基硫過氧化酶（glutathione peroxidase）、過氧化氫酶（catalase）、超氧化歧解酶（superoxide dismutase, SOD）的發炎防禦機制也被證實與氣喘有關 [12]。

在 guaninae 鹼基之 C-8 上產生 hydroxylation，是 ROS 最容易引起的氧化性 DNA 傷害 [13,14]，進一步地在 DNA 複製及修補時產生鹼基配對上的錯誤，特別是 G → T 的取代 [15]；藉由 exonuclease 修補後的 DNA 可以形成水溶性 8-hydroxyl-2′-deoxyguanosine（8-OHdG）。但是，氧化性 DNA 傷害指標 8-OHdG 是否與兒童氣喘之發生具有相關性，目前仍未被清楚地探討；相信比較氣喘兒童與非氣喘兒童其尿液 8-OHdG 之表現，將有助於解釋細胞氧化性 DNA 傷害與氣喘致病機制的關連。因此，本研究之預期假說爲氣喘兒童較非氣喘兒童具有較高的 ETS 暴露程度；而氣喘兒童較非氣喘兒童具有較高的尿液 8-OHdG 濃度。

材料與方法

一、病例確認與對照選取

本研究從中山醫學大學附設醫院小兒科選取 35 名 4-12 歲氣喘兒童爲病例組。病例是經由臨床醫師診斷，而氣喘之定義是根據世界衛生組織 [16] 所規定具有以下現象者：(a) 呼吸道阻塞、(b) 呼吸道有咻咻哮喘聲、(c) 會因氣管擴張劑或 corticosteriods 而改變呼吸道阻塞、(d) 支氣管加強反應、(e) 發炎作用會引發嗜鹼性白血球、嗜伊紅性白血球、肥大細胞、T 淋巴球及許多細胞反應。

本研究以 1：1 之病例與對照的比例進行配對，對照是選取與病例同年齡之非氣喘兒童；合計研究樣本數共計 70 名。

二、流行病學資料

研究對象的個人特徵資料，在獲取所有參與者的同意書後，經由面對面的問卷訪視所收集。結構式問卷所涵蓋的問題包括：人口學特質、生活型態如與兒童共同生活之家庭成員抽菸狀態、室內其它污染源如燒香、潮濕度、是否飼養寵物等，以及氣喘的家族史。研究對象的家庭成員抽菸狀態包括每天抽菸支數及抽菸年數；兒童室內 ETS 累積暴露量是以每天平均暴露的香菸包年（pack-year）來計算，亦即所有與兒童共同生活之家庭成員其每天個別的抽菸包數乘以與受測兒童共同生活至氣喘發病之年數的總合；若爲未發病的兒童，則爲所有與兒童共同生活之家庭成員其每天個別的抽菸包數乘以與受測兒童共同生活至研究截止日之年數。住家的潮濕程度在最近一年內符合以下條件之一者即定義爲潮濕：可以看見家戶內部表面具有黴菌滋生、家戶內積水、或漏水。氣喘家族病史，則是以受測者之一等親家族具有氣喘來加以定義。

三、尿液氧化性DNA傷害標記8-OHdG

尿液的採集是兒童於門診時收集當次尿液，在獲取所有研究對象的尿液 15 毫升後，將尿液樣本立刻放入冰筒中，帶回實驗室儲存於 -70°C 冰箱。使用酵素連結免疫吸附分析法（enzyme-linked immunosorbent assay, ELISA; Japan Institute for the Control of Aging, Japan）方法來偵測尿液細胞中的氧化性 DNA 傷害標記 8-OHdG[17]；偵測極限爲 0.5-200 ng/ml。以 50 ml 的尿液，加入事先黏附抗體的酵素免疫分析盤孔內，然後在 37°C 下培養一小時。清洗掉後，加入 50 ml 的二次多株抗體於每個孔內，室溫下培養一小時，然後再度洗掉；此時剩餘的二次抗體已經與 horseradish peroxidase (HRP)-conjugated anti-rabbit IgG 鍵結，再加入受質（TM blue, 3,3′, 5,5′-tetramethylbenzidine）後即可產生顏色。以 450 nm 波長在 ELISA reader 中偵測每個樣本的吸光值，並與標準樣本所得的吸光值作比對後，來決定樣本中 8-OHdG 的濃度。檢量線之相關係數 (r) 需大於 0.995 且每個尿液樣本重複測量之變異係數需小於 10%，才能接受。

尿液中肌酸酐（creatinine）的測量是做爲尿液體積校正之用，此部份則委由中山醫學大學附設醫院檢驗醫學部測量。校正後的 8-OHdG 濃度（ng/mg creatinine）爲

尿液中 8-OHdG 濃度（ng/ml）除以肌酸酐濃度（mg/dl）再乘以 100。

四、統計分析

計算孩童性別、父母親教育程度、一等親氣喘家族史、父母親抽菸習慣、二手菸暴露、室內其他污染狀態如家中從事毛織的工作、燒香、潮濕度、是否飼養寵物、以及孩童的臥房是否有蟑螂等因子，對於氣喘發生的勝算比（odds ratio, OR）以及 95% 信賴區間（95% confidence interval, C.I.）；同時以無母數分析檢定兒童尿中之 8-OHdG 濃度於病例及對照組間是否也有不同。隨後，多變項迴歸模式被執行來檢驗尿液 8-OHdG 濃度與不同因子之間的相關；迴歸係數以及標準誤也被計算。

結　果

所有研究對象的基本特徵，呈現於表 1。研究對象的性別爲男孩（82.9% vs. 48.6%, OR = 5.1; 95% C.I. = 1.7-15.4）、父親教育程度爲高中及以上（37.1% vs. 8.6%, OR = 6.3; 95% C.I. = 1.6-24.8）、母親教育程度爲高中及以上（34.3% vs. 2.9%, OR = 17.7; 95% C.I. = 2.2-146.0）、以及具有一等親氣喘家族史（22.9% vs. 2.9%, OR = 10.1; 95% C.I. = 1.2-85.6），在病例組中的比例皆明顯高於對照組。

環境因子在兒童氣喘之病例與對照組間的分佈，呈現在表 2。在病例與對照兩組間，父母親抽菸的習慣並未具有統計顯著差異；而研究對象之二手菸暴露量大於（含）一包年以上者，佔病例組的 42.9%，相較於對照組 25.7%，具有較高的勝算比（OR = 2.2, 95% C.I. = 0.8-6.0）。而研究對象家中是否從事毛類織品的工作、是否飼養寵物、孩童的臥房是否有看過蟑螂、家中是否常燒香拜拜、孩童臥房的牆壁是否會長霉等環境因子，在病例與對照組間的分佈並未呈現統計顯著差異。

所有研究對象的 8-OHdG 濃度在各變項之分佈，呈現在表 3。病例組相較於對照組具有顯著較高的 8-OHdG 濃度（77.4 vs. 17.8 ng/ml creatinine, $P = 0.004$; Wilcoxon rank sum test），而男孩也相較於女孩具有較高的 8-OHdG 濃度（58.7 vs. 26.2, $P = 0.008$）；特別在對照組中，此濃度差異表現達到統計顯著程度（21.1 vs. 14.6, $P = 0.02$）。孩童具有一等親氣喘家族史者也相較於無一等親氣喘家族史者，具有明顯較高的 8-OHdG 濃度（90.0 vs. 41.3, $P = 0.06$）。此外，不管是在病例組或是對照組中父母親是否具有抽菸的習慣，孩童的尿液 8-OHdG 濃度並沒有差異；而在對照組中，二手菸暴露量大於一包年者相較於小於一包年者，具有較高的尿液 8-OHdG 濃度，同樣地未達統計顯著性（20.0 vs. 17.0 ng/ml creatinine, $P = 0.21$）。家中有無飼養寵物、孩童的臥房有否看過蟑螂、家中燒香拜拜的頻率、以及孩童臥房的牆壁是否會長霉的狀況，並不會導致孩童的尿液 8-OHdG 濃度產生差異。

在隨後的多變項迴歸分析中（表 4），男孩（$P < 0.001$）、以及一等親氣喘家族史（$P < 0.001$）與對照健康兒童其尿液中 8-OHdG 濃度的增加，具有統計上的顯著相關；而每增加一包年的二手菸暴露量，也隨之增加 1.4 ng/mg creatinine 的 8-OHdG 濃度（$P < 0.001$）。而性別、一等親氣喘家族史與二手菸暴露量，分別可解釋對照健康兒童其尿液中 8-OHdG 濃度 57%、22%、21% 的變異。

討論與結論

本研究發現，氣喘兒童較非氣喘兒童之尿液 8-OHdG 濃度有顯著較高的表現。許多的證據建議氧化壓力（oxidant stress）可導致呼吸道系統的發炎與組織傷害，進而導致免疫調節的改變 [11,12]，因而個體就有可能發展出氣喘。而在本研究中，氣喘兒童較非氣喘兒童之尿液 8-OHdG 濃度有顯著較高的表現，應該可以代表特定發炎組織的表現；雖然，尿液細胞的氧化性 DNA 傷害可能代表著全身的氧化反應之表現。在我們的研究中，所有的氣喘研究對象是不具其他症狀的兒童，所以應可忽略其他組織傷害的干擾。因此，可能可以解釋氣喘兒童其尿液具有較高濃度的氧化性 DNA 傷害指標的一個理由是，呼吸道的發炎可能刺激身體的防禦系統產生細胞激素；一些細胞激素可以產生大量的 ROS[18,19]，並且釋放出大量的發炎細胞到血流中，運送到其他組織。

先前研究已經證實二手菸的暴露與氣喘間的關連性，但是二手菸暴露對於兒童氣喘發生的致病機轉，至今仍不清楚。香菸中包含大量的自由基，而 ROS 可以造成細胞 DNA 結構改變。流行病學研究指出，抽菸者其尿液中 8-OHdG 濃度較未暴露者顯著增加 [20]；一項動物實驗也顯示，暴露在二手菸的大鼠較未暴露的大鼠會產生較高的 8-OHdG 含量 [21]。在我們的研究中，具有二手菸暴露的兒童具有較高的危險可發展成氣喘，雖然統計未達顯著性。而每增加一包年的二手菸暴露量，健康對照兒童也隨之增加 1.4 ng/mg creatinine 的 8-OHdG 濃度；但是在氣喘兒童中，並未見具有二手菸暴露者表現出較高的 8-OHdG 濃度。這可能是反映著個體接受到二手菸暴露可逐漸地導致細胞的氧化傷害，但是一旦發展成氣喘後，體內氧化傷害即持續的表現出來，此時就不受先前的致病因素所影響了。

在我們的研究中，男孩相較於女孩也具有較高危險的氣喘發生危險，這與 Arshad 等人 [22] 於英國所執行的一項長期追蹤研究的結果一致；並且，我們的觀察也發現到，氣喘與非氣喘的男孩也都具有較高的尿液 8-OHdG 濃度。此種現象可能如同 Loft 等人 [23] 先前

表 1 研究對象之基本特性分佈

變　項	病例組	對照組	
	個數 = 35	個數 = 35	勝算比 (95%信賴區間)
性別			
男孩	29 (82.9%)	17 (48.6%)	5.1 (1.7-15.4)
女孩	6 (17.1%)	18 (51.4%)	1.0
父親			
高中及以上程度	13 (37.1%)	3 (8.6%)	6.3 (1.6-24.8)
高中以下程度	22 (62.9%)	32 (91.4%)	1.0
母親			
高中及以上程度	12 (34.3%)	1 (2.9%)	17.7 (2.2-146.0)
高中以下程度	23 (65.7%)	34 (97.1%)	1.0
一等親氣喘家族史			
是	8 (22.9%)	1 (2.9%)	10.1 (1.2-85.6)
否	27 (77.1%)	34 (97.1%)	1.0

表 2 環境因子在研究對象間的分佈

變項	病例組	對照組	
	個數 = 35	個數 = 35	勝算比 (95%信賴區間)
父親有抽菸的習慣			
是	14 (40.0%)	17 (48.6%)	0.7 (0.3-1.8)
否	21 (60.0%)	18 (51.4%)	1.0
母親有抽菸的習慣			
是	3 (8.6%)	0 (0.0%)	7.7 (0.4-153.8)
否	32 (91.4%)	35 (100%)	1.0
二手菸暴露量			
≥1包年	15 (42.9%)	9 (25.7%)	2.2 (0.8-6.0)
<1包年	20 (57.1%)	26 (74.3%)	1.0
家中從事毛類織品的工作			
是	2 (5.7%)	4 (11.4%)	0.5 (0.1-2.8)
否	33 (94.3%)	31 (88.6%)	1.0
家中飼養寵物			
是	9 (25.7%)	8 (22.9%)	1.2 (0.4-3.5)
否	26 (74.3%)	27 (77.1%)	1.0
孩童的臥房有看過蟑螂			
是	13 (37.1%)	12 (34.3%)	1.1 (0.4-3.0)
否	22 (62.9%)	23 (65.7%)	1.00
家中常燒香拜拜			
幾乎每天	4 (11.4%)	2 (5.7%)	2.3 (0.4-14.1)
初一、十五及特殊節日	12 (34.3%)	11 (31.4%)	1.3 (0.5-3.5)
平均每個月不到一次	19 (54.3%)	22 (62.9%)	1.0
孩童臥房的牆壁會長霉			
平常就會	2 (5.7%)	3 (8.6%)	0.6 (0.1-4.2)
常下雨的季節才會	5 (14.3%)	5 (14.3%)	1.0 (0.3-3.7)
否	28 (80.0%)	27 (77.1%)	1.0

表 3 8-OHdG 濃度在兒童氣喘之病例組與對照組間的分佈

變項	病例組		對照組		全部	
	個數	平均值 ± 標準差	個數	平均值 ± 標準差	個數	平均值 ± 標準差
全部	35	77.4 ± 105.0	35	17.8 ± 11.6	70	47.6 ± 80.0
性別						
男孩	29	80.8 ± 112.8	17	21.1 ± 8.7	46	58.7 ± 93.8
女孩	6	61.0 ± 56.5	18	14.6 ± 13.4	24	26.2 ± 35.3
		$P = 0.88$		$P = 0.02$		$P = 0.008$
父親有抽菸的習慣						
是	14	58.6 ± 91.5	17	17.1 ± 8.3	31	35.9 ± 64.0
否	21	90.0 ± 113.5	18	18.3 ± 14.3	39	56.9 ± 90.4
		$P = 0.28$		$P = 0.86$		$P = 0.41$
母親有抽菸的習慣						
是	3	16.6 ± 4.2	0	-	3	14.6 ± 4.2
否	32	83.3 ± 108.0	35	17.8 ± 11.6	67	49.1 ± 81.5
		$P = 0.16$				$P = 0.34$
二手菸暴露量						
≥1包年	15	53.2 ± 87.9	9	20.0 ± 6.9	24	40.8 ± 70.7
<1包年	20	95.6 ± 114.9	26	17.0 ± 12.9	46	51.5 ±85.0
		$P = 0.18$		$P = 0.21$		$P = 0.78$
家中從事毛類織品的工作						
是	2	18.0 ± 16.1	4	13.5 ± 10.1	6	15.0 ±10.9
否	33	81.0 ± 107.1	31	18.3 ± 11.9	64	50.6 ± 83.0
		$P = 0.31$		$P = 0.49$		$P = 0.14$
家中飼養寵物						
是	9	93.4 ± 106.1	8	14.4 ± 9.1	17	56.2 ± 85.6
否	26	71.9 ± 106.1	27	18.8 ± 12.3	53	44.8 ± 78.8
		$P = 0.31$		$P = 0.34$		$P = 0.80$
孩童的臥房有看過蟑螂						
是	13	74.8 ± 97.8	12	16.4 ± 15.9	25	46.8 ± 76.1
否	22	79.0 ± 111.2	23	18.5 ± 9.0	45	48.0 ± 83.0
		$P = 0.91$		$P = 0.20$		$P = 0.54$
家中常燒香拜拜						
幾乎每天	4	52.4 ± 59.5	2	22.2 ± 9.0	6	42.3 ± 48.8
初一、十五及特殊節日	12	92.4 ± 120.3	11	20.1 ± 14.7	23	57.8 ± 93.3
平均每個月不到一次	19	73.2 ± 105.2	22	16.2 ± 10.3	41	42.6 ± 76.6
		$P = 0.61$		$P = 0.62$		$P = 0.43$
孩童臥房的牆壁會長霉						
平常就會	2	54.5 ± 35.6	3	19.3 ± 7.3	5	33.4 ± 26.8
常下雨的季節才會	5	83.8 ± 108.5	5	24.7 ± 21.8	10	54.3 ± 80.0
否	28	77.9 ± 109.7	27	16.3 ± 9.4	55	47.7 ± 83.8
		$P = 0.78$		$P = 0.63$		$P = 0.60$
一等親氣喘家族史						
是	8	93.8 ± 128.8	1	59.7	9	90.0 ± 121.0
否	27	72.6 ± 99.2	34	16.5 ± 9.2	61	41.3 ± 71.4
		$P = 0.68$		$P = 0.11$		$P = 0.06$

表 4 8-OHdG 濃度在兒童氣喘之對照組中的簡單直線迴歸模式

變項	8-OHdG濃度		*P*值	部分解釋比例
	迴歸係數	標準誤		
截距	7.3	2.0	< 0.001	-
性別：男 vs. 女	13.5	2.6	< 0.001	57%
一等親氣喘家族史：有 vs. 無	52.4	7.1	< 0.001	22%
二手菸暴露量：每增加一包年	1.4	0.4	< 0.001	21%
R^2	0.69			

所建議的，男孩相較於女孩有較高的基礎代謝率，體內能量消耗較快，相對地細胞內氧化的速度增加；此外，也可能反映著男孩相較於女孩，較容易暴露到可導致細胞氧化性 DNA 傷害的物種，因於容易發展出氣喘。同樣地，具有一等親氣喘家族史者與兒童氣喘發生具有顯著相關，具有一等親氣喘家族史者也具有較高的尿液 8-OHdG 濃度。氣喘的致病機轉，除了環境因素外，家族因素也是重要原因之一；若有異位性疾病之家族史，則有較高的危險性會發生氣喘 [24]，並且研究亦指出同卵雙胞胎得到異位性疾病較異卵雙胞胎有更高之一致性 [24,25]；而 Harris 等人 [26] 所執行的雙胞胎研究中，則認爲有 75% 的氣喘變異性是由基因所影響。因此，一等親氣喘家族史與兒童氣喘發生的相關，可能是暴露於可產生氧化性 DNA 傷害的共同環境因素，或是與氧化傷害機制上相關的遺傳因素有關;但仍須進一步的研究證實。

此外，研究對象家中是否從事毛類織品的工作、家中是否飼養寵物、孩童的臥房是否有看過蟑螂、家中是否常燒香拜拜、孩童臥房的牆壁是否會長霉等，在病例與對照組間的分佈並未呈現統計顯著差異；並且具有這些特徵的研究對象其尿液 8-OHdG 濃度並未具有顯著增加。這可能表示這些特徵可能與兒童的氧化性 DNA 傷害機制無關。而在我們的研究中，僅有 70 名研究對象被執行分析，樣本數目較少限制了我們的檢定力。此外，Pilger 等人 [27] 的研究發現健康成人一次的尿液檢測無法反映個體 8-OHdG 濃度變化，我們的研究中納入 4-12 歲的兒童，尿液的採集是於門診時收集當次尿液，因此，我們偵測的 8-OHdG 濃度可能會有低估的情形。

總結來說，本研究發現氣喘兒童較非氣喘兒童之尿液 8-OHdG 濃度有顯著較高的表現；這項結果反映著細胞的氧化性 DNA 傷害可能對於兒童氣喘的發生扮演重要的角色。

參考文獻

1. Braunwald E, Fauci AS, Kasper DL, Hauser SL, Longo DL, Jameson JL. Harrison's Principles of Internal Medicine, 15th ed. New York: McGraw-Hill Medical Publishing, 2001; 1456-63.
2. Park JH, Gold DR, Spiegelman DL, Burge HA, Milton DK. House dust endotoxin and wheeze in the first year of life. Am J Respir Crit Care Med 2001;163:322-8.
3. Chung KF. Non-invasive biomarkers of asthma. Pediatr Pulmonol - Supplement 1999;18:41-4.
4. Pedersen PA, Weeke ER. Epidemiology of asthma in Denmark. Chest 1987;91(6 Suppl):107-14.
5. Chen CF, Wu KG, Hsu MC, Tang RB. Prevalence and relationship between allergic diseases and infectious diseases. J Microbiol Immunol Infect 2001;34:57-62.
6. Cunningham J, O'Connor GT, Dockery DW, Speizer FE. Environmental tobacco smoke, wheezing, and asthma in children in 24 communities. Am J Respir Crit Care Med 1996;153:218-24.
7. Cook DG, Strachan DP, Carey IM. Health effects of passive smoking. 9. Parental smoking and spirometric indices in children. Thorax 1998;53:884-93.
8. Rahman I, MacNee W. Role of oxidants/antioxidants in smoking-induced lung diseases. Free Radic Biol Med 1996; 21:669-81.
9. Pryor WA, Stone K. Oxidants in cigarette smoke. Radicals, hydrogen peroxide, peroxynitrate, and peroxynitrite. Ann N Y Acad Sci 1993;686:12-27.
10. Janoff A, Pryor WA, Bengali ZH. NHLBI workshop summary. Effects of tobacco smoke components on cellular and biochemical processes in the lung. Am Rev Respir Dis 1987;136:1058-64.
11. Halliwell B, Aruoma OI. DNA damage by oxygen-derived species. Its mechanism and measurement in mammalian systems. FEBS Lett 1991;281:9-19.
12. Smith LJ, Shamsuddin M, Sporn PH, Denenberg M, Anderson J. Reduced superoxide dismutase in lung cells of patients with asthma. Free Radic Biol Med 1997;22:1301-7.
13. Breen AP, Murphy JA. Reactions of oxyl radicals with DNA. Free Radic Biol Med 1995;18:1033-77.
14. Moriya M. Single-stranded shuttle phagemid for mutagenesis studies in mammalian cells: 8-oxoguanine in DNA induces targeted G.C-->T.A transversions in simian kidney cells. Proc Natl Acad Sci U S A 1993;90:1122-6.
15. Cheng KC, Cahill DS, Kasai H, Nishimura S, Loeb LA. 8-Hydroxyguanine, an abundant form of oxidative DNA damage, causes G→T and A→C substitutions. J Biol Chem 1992;267:166-72.
16. Anonymous. Global strategy for asthma management and prevention NHLBI/WHO workshop report. Global initiative for asthma. Washington, DC: NHLBI, 1994.
17. Wong RH, Yeh CY, Hsueh YM, Wang JD, Lei YC, Cheng TJ. Association of hepatitis virus infection, alcohol

consumption and plasma vitamin A levels with urinary 8-hydroxydeoxyguanosine in chemical workers. Mutat Res 2003;535:181-6.
18. Ohba M, Shibanuma M, Kuroki T, Nose K. Production of hydrogen peroxide by transforming growth factor-beta 1 and its involvement in induction of egr-1 in mouse osteoblastic cells. J Cell Biol 1994;126:1079-88.
19. Kayanoki Y, Fujii J, Suzuki K, Kawata S, Matsuzawa Y, Taniguchi N. Suppression of antioxidative enzyme expression by transforming growth factor-beta 1 in rat hepatocytes. J Biol Chem 1994;269:15488-92.
20. Lee BM, Lee SK, Kim HS. Inhibition of oxidative DNA damage, 8-OHdG, and carbonyl contents in smokers treated with antioxidants (vitamin E, vitamin C, beta-carotene and red ginseng). Cancer Lett 1998;132:219-27.
21. Maehira F, Miyagi I, Asato T, Eguchi Y, Takei H, Nakatsuki K, et al. Alterations of protein kinase C, 8-hydroxydeoxyguanosine, and K-ras oncogene in rat lungs exposed to passive smoking. Clin Chim Acta 1999;289:133-44.
22. Arshad SH, Stevens M, Hide DW. The effect of genetic and environmental factors on the prevalence of allergic disorders at the age of two years. Clin Exp Allergy 1993;23: 504-11.
23. Loft S, Vistisen K, Ewertz M, Tjonneland A, Overvad K, Poulsen HE. Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. Carcinogenesis 1992; 13:2241-7.
24. Duffy DL, Martin NG, Battistutta D, Hopper JL, Mathews JD. Genetics of asthma and hay fever in Australian twins. Am Rev Respir Dis 1990;142:1351-8.
25. Hopp RJ, Bewtra AK, Watt GD, Nair NM, Townley RG. Genetic analysis of allergic disease in twins. J Allergy Clin Immunol 1984;73:265-70.
26. Harris JR, Magnus P, Samuelsen SO, Tambs K. No evidence for effects of family environment on asthma. A retrospective study of Norwegian twins. Am J Respir Crit Care Med 1997;156:43-9.
27. Pilger A, Germadnik D, Riedel K, Meger-Kossien I, Scherer G, Rüdiger HW. Longitudinal study of urinary 8-hydroxy-2'-deoxyguanosine excretion in healthy adults. Free Radic Res 2001;35:273-80.

Differences in urinary 8-hydroxy-2′-deoxyguanosine levels between asthmatic and non-asthmatic children

Ming-Fuu Wang[1], Ko-Huang Lue[2], Pi-I Chang[3], Ming-Lin Hsu[3], Tsun-Yen Wang[3], Ruey-Hong Wong[3,*]

[1] *Division of Allergy, Immunology, and Rheumatology, Department of Pediatrics, Tungs' Taichung Metroharbor Hospital*
[2] *Department of Pediatrics, Chung-Shan Medical University Hospital*
[3] *Department of Public Health, Chung Shan Medical University*

Received: Feb. 1, 2012; Accepted: Mar. 29, 2013

Abstract

Background: Asthma induced by exposure to environmental tobacco smoke (ETS) may be associated with oxidative DNA damage caused by reactive oxygen species. However, the association between the urinary oxidative DNA damage marker 8-hydroxy-2′-deoxyguanosine (8-OHdG) and childhood asthma has not been clearly elucidated.
Methods: This study recruited 35 asthmatic children as the case group and 35 non-asthmatic children as the control group. Information regarding personal characteristics of the study subjects was obtained during face-to-face interviews. The term "pack-years" was used as an indicator of cumulative ETS levels of a child, and it was defined as the number of packs of cigarettes smoked daily by the subjects' family members who were living together multiplied by the duration of exposure to ETS. Levels of 8-OHdG were measured by enzyme-linked immunosorbent assay (ELISA).
Results: The case group had a higher proportion of boys and those with a family history of asthma than the control group. Compared with control group, the case group had significantly higher levels of 8-OHdG (77.4 vs. 17.8 ng/ml creatinine, $P = 0.004$). In the control group of non-asthma boys ($P < 0.001$) also had significantly increased 8-OHdG level. Especially, a pack year of ETS exposure dose could increase 1.4 ng/mg creatinine ($P < 0.001$) of urinary 8-OHdG concentration in those non-asthma group.
Conclusion: Oxidative DNA damage in the cell may play an important role in the development of childhood asthma.

Key words: Environmental tobacco smoke, asthma, 8-hydroxy-2′-deoxyguanosine

*Correspondence to: Dr. Ruey-Hong Wong, Department of Public Health, Chung Shan Medical University, No. 110 Chien-Kuo N Rd., Sec. 1, Taichung, 40201, Taiwan (R.O.C.)

Tungs' Medical Journal 7 (2013) 18-25

Original Article

急診頸椎電腦斷層中聲帶萎縮的盛行率

陳建志[1,2,3,*]　沈里國[3]　王興萬[4,5]　陳登郎[6]　湯耀貿[7]

中國醫藥大學附設醫院　神經部[1]
中國醫藥大學[2]
台北醫學大學暨雙和醫院　影像醫學部[3]　耳鼻喉科[4]
台北醫學大學臨床醫學研究所[5]
吉豐耳鼻喉科診所[6]
湯耀貿耳鼻喉科診所[7]

受文日期：民國 101 年 5 月 7 日；接受刊載：民國 102 年 7 月 24 日

摘要

背景及目的：聲帶萎縮普遍認為與老化有關，在老年人應該較多，目前有關之研究均著眼於耳鼻喉科門診患者。本文特以回顧性影像學的方法，試圖在曾接受過急診頸椎電腦斷層的患者群中找出一般族群的狀況。

方法：共計 233 名患者（男 127，女 106）納入本研究，年齡 46.0±19.2（平均值 ± 標準差）歲，曾於 2010 年 9 月至 2011 年 4 月間接受急診頸椎電腦斷層。運用表面數位重建以虛擬喉鏡辨認是否具聲帶萎縮，接著利用 2 維多層面數位重建找到恰位於兩側杓骨尖端與前聯合後端的聲門平面，測量前聯合厚度、兩側膜性聲帶平均直線長度及夾角，以及聲門氣道面積。統計分析採用卡方檢定與雙尾 t 檢定，α 值設為 .01。

結果：共 14.2%（n=33）具有聲帶萎縮，其中僅 12.1%（4 of 33）為老年人（≥ 65 歲）。不論男性或女性，有無聲帶萎縮者在老年或非老年人間並無差異（p= .8232 & .1337）。聲帶萎縮對女性的影響較男性明顯；在前者，前聯合厚度較小（$p=7.66 \times 10^{-6}$），兩側膜性聲帶平均直線長度較長（p= .0002），聲門氣道面積較大（p= .0006）；在後者，僅兩側膜性聲帶平均直線長度較長（p= .0097）

結論：聲帶萎縮並非在老年人較多，或許僅是一種解剖學上的異常，不全然是老化所致。

關鍵詞：聲帶萎縮、虛擬喉鏡、表面數位重建、2維多層面數位重建、頸椎電腦斷層

前　言

普遍認爲理學檢查（或間接喉鏡）只要發現聲帶呈現弓形變化（bowing）及聲門閉合不全（glottic insufficiency），造成氣音、說話容易累、口乾、喉嚨有痰感…等症狀時即可確診爲聲帶萎縮（vocal fold atrophy）[1,2]，目前很多研究認爲聲帶萎縮主要導因於聲帶甲杓肌（thyroarytenoid muscles）的萎縮 [3,4]。根據陳等之研究 [5]，聲帶萎縮佔所有慢性咳嗽患者之 61.7%。根據 Takano 等之研究 [2]，聲帶萎縮佔音聲特別門診之全數老年患者（≥ 65 歲）之 20%，其中 65% 爲男性，35% 爲女性。根據李等之研究 [6]，聲帶萎縮佔所有門診接受間接喉鏡檢查患者之 0.7%，全是女性。可見，聲帶萎縮究竟是男性多還是女性多？是否比較盛行於老年人？目前仍未有定論，然而，以上研究均著眼於耳鼻喉科門診患者，其實很難反應出一般族群確實的狀況。爲了不違背人體試驗倫理議題，本文結合科技設備以相對非侵入方式執行研究，特回顧急診頸椎電腦斷層，運用表面數位重建（surface rendering）及 2 維多層面數位重建（multiplanar reformatting）等影像後處理技術，找出聲帶萎縮者，並進行相關的統計與分析。

* 通訊作者：陳建志醫師　中國醫藥大學附設醫院　神經部
40447 台中市北區育德路 2 號

材料及方法

研究對象

於 2010 年 9 月 至 2011 年 4 月 間， 共 計 272 名 急

診患者因疑似頸椎外傷就診於北部某區域教學醫院，必須接受頸椎電腦斷層（cervical spine computed tomography）。在排除使用氣管內管（n=16）、閉氣或吞嚥動作導致聲門閉合（n=13）、患者躁動造成假影（n=2）、鼻胃管（n=1）及未滿 18 歲者（n=7）後，剩餘 233 名患者（男 127，女 106）納入本研究，年齡 46.0±19.2（平均值 ± 標準差）（範圍 18~94）歲，其中有 221 名無頸椎急症，另 12 名分別診斷爲頸椎骨折（n=9）、頸椎脫臼（n=2）、顏面骨折（n=1）及前方頸部（第 6 區）外傷（n=1）。

頸椎電腦斷層掃描

本院急診使用 16 切面螺旋電腦斷層掃描儀（BrightSpeed TM Elite，GE Healthcare，美國）。受檢者均採仰臥姿，頭先進入，正常呼吸，除非是合併胸部或腹部電腦斷層必須同時配合閉氣。從顱底到胸骨凹處進行螺旋狀連續掃瞄（切片厚度 0.625 mm，間隔 1.0 mm，每圈 1 秒，120 KV，275 mA），接著把掃描後的立體像素組成連續軸狀面的影像（切片厚度 1.25 mm，間隔 1.0 mm，重疊比 0.938，視野 16 cm，解析度 512×512），儲存於數位醫學影像閱片系統（EBM technologies: safety-critical system，EBM Technologies Incorporated，台灣）。

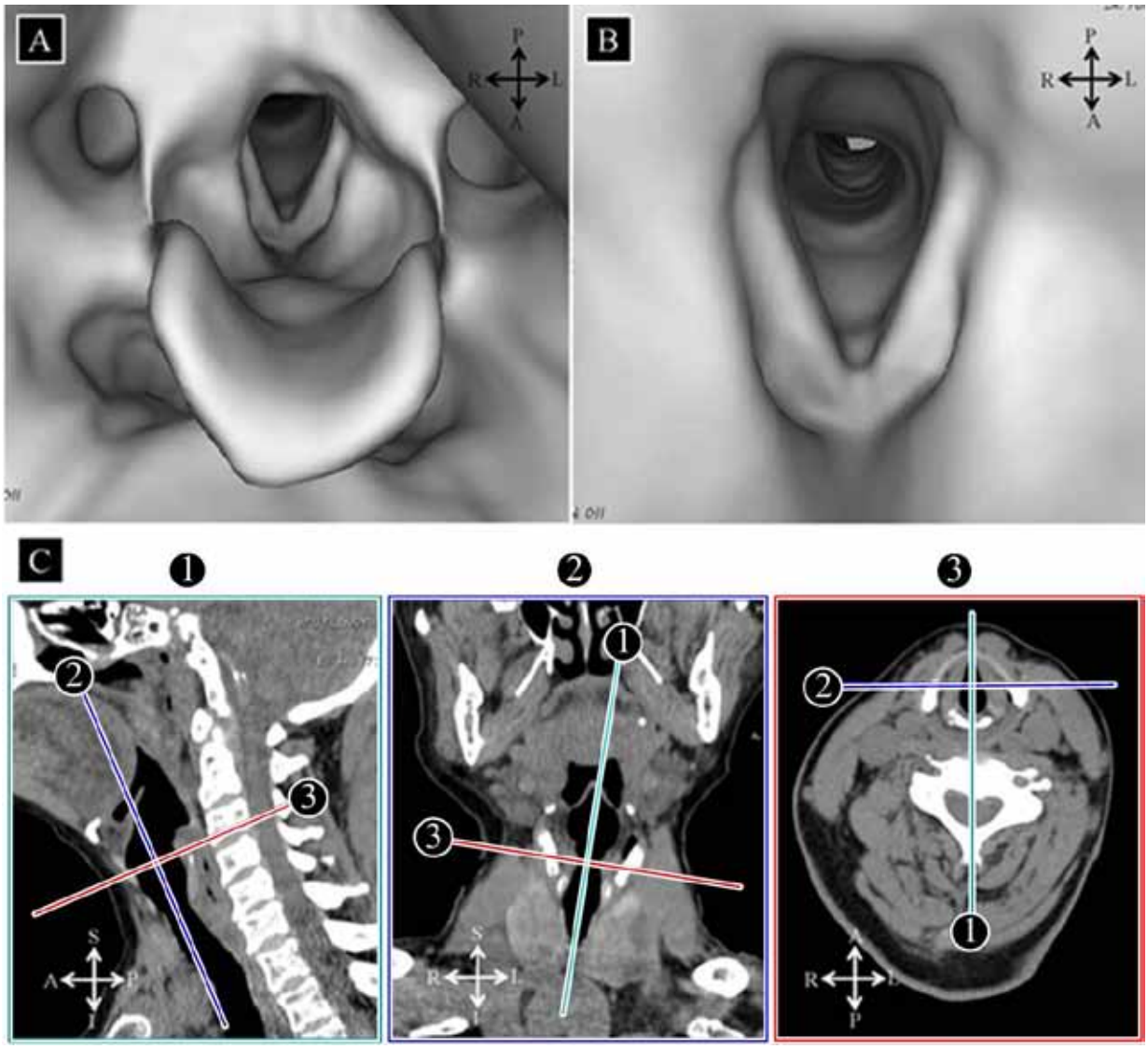


圖 1　一 78 歲女性於交通意外後急診於本院，因疑似頸椎外傷接受頸椎電腦斷層檢查，結果無異常發現，建議保守治療。利用表面數位重建呈現虛擬喉鏡 (A)，檢視聲帶狀況 (B)。利用 2 維多層面數位重建呈現恰位於前聯合最後端、兩側杓狀軟骨尖端的軸狀聲門切面 (視野 25 cm)(C)。

後置影像處理

把儲存的立體像素輸出到後置影像處理系統（Advantage Workstation 4.4，GE Healthcare，美國），選擇導覽功能（navigation）呈現喉部表面數位重建後虛擬喉鏡（virtual laryngoscopy）影像（圖 1A），審視聲門是否因閉氣或吞嚥動作而關閉，把視野中心對準聲門氣道中心（圖 1B）。接著開啓 2 維多層面數位重建功能（圖 1C），設定在軟組織視窗（窗幅值 350 HU，中心值 45 HU）以分辨甲狀軟骨、杓狀軟骨、膜性聲帶與聲門氣道。

在矢狀切面（1 號框）下滾動視窗滾輪，找到聲門氣道前後徑最大處，調整代表軸狀切面（3 號框）的 3 號線條，設法讓其滾動視窗滾輪時，可以滑過前聯合後端與兩側杓狀軟骨尖端；在冠狀切面（2 號框）下調整代表矢狀切面（1 號框）的 1 號線的角度，設法讓其位於聲門氣道正中線，設法讓其在滾動視窗滾輪時可以滑過會厭尖端，接著調整代表軸狀切面（3 號框）的 3 號線，設法讓其在滾動視窗滾輪時可以通過兩側杓骨尖端；在軸狀切面（3 號框）下調整代表矢狀切面（1 號框）的 1 號線，設法讓其在滾動視窗滾輪時均位於聲門氣道正中線，調整代表冠狀切面（2 號框）的 2 號線，設法讓其在滾動視窗滾輪時剛好通過兩側杓骨尖端，最後，在 2 號線通過兩側杓骨尖端時，把該軸狀切面（2 號框）定格。

聲門參數

把已定格之軸狀聲門切面放大（視野 6.5 cm）（圖 2）。前聯合厚度定義爲聲門最前端至甲狀軟骨後緣的距離（a 線）；右側膜性聲帶直線長度定義爲右側杓骨尖端到前聯合最後端的直線距離（b 線），依此類推定義左側膜性聲帶直線長度（c 線）；兩側膜性聲帶直線長度比例定義爲右側除以左側膜性聲帶直線長度後所得之值，而兩側膜性聲帶平均直線長度則爲兩側膜性聲帶直線長度之平均值；兩側膜性聲帶直線夾角定義爲兩側杓骨尖端連到前聯合最後端（弧形虛線）所形成之夾角。聲門氣道面積定義爲在此聲門平面上，聲門氣道外緣所圍成之面積（虛線圈）。

測量方法

選取工具列中的「長度」小圖示，在軸狀聲門切面圖上，按住滑鼠左鍵，將十字游標脫曳至欲測量之兩目標點，輕按滑鼠右鍵，就會呈現這兩目標點間的距離。選取「夾角」小圖示，按住滑鼠左鍵，將十字游標脫曳至欲測量之三目標點，設法讓第二目標點位於預測量之夾角端點，輕按滑鼠右鍵，就會呈現兩條直線夾角的角度。選取「多邊形」小圖示，按住滑鼠左鍵，沿著聲門氣道外緣描繪一圈，讓多邊形之邊緣線落在聲門氣道外緣，輕按滑鼠右鍵，就會呈現該多邊形的面積。

成年族群定義

65 歲以上爲老年人，18~64 歲爲一般成年人。

聲帶萎縮定義

虛擬喉鏡見到至少一側聲帶甲杓肌處有「凹陷」（圖 3A&B，實心箭號），而該「凹陷」足以在聲門平面軸狀切面上造成至少一側聲帶韌帶外側之「空洞」（圖 3A&B，空心箭號）。爾後，具聲帶萎縮者簡稱爲「陽性者」，反之簡稱爲「陰性者」。

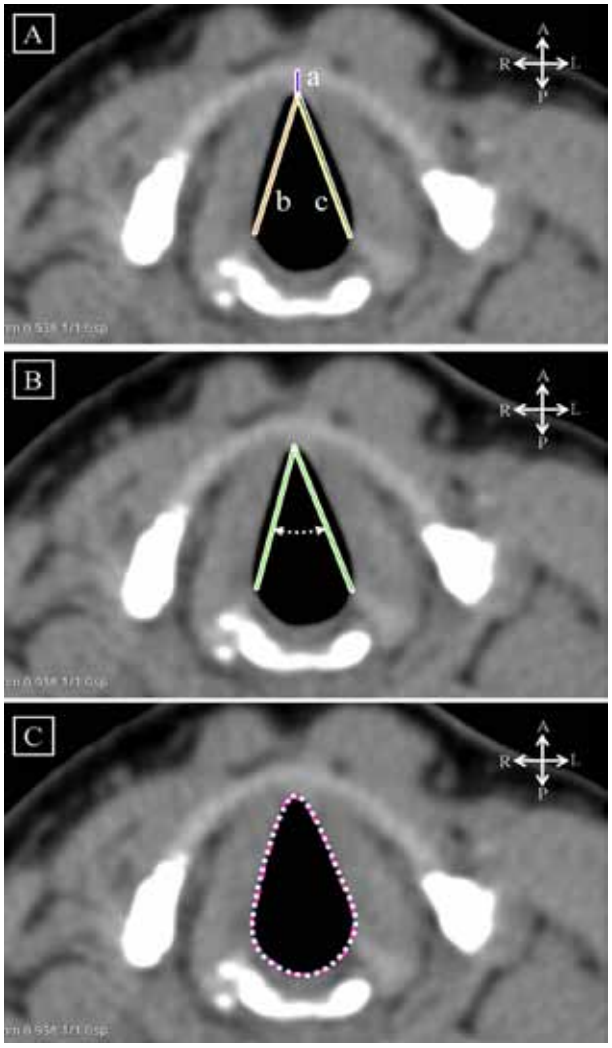


圖 2　放大此軸狀聲門切面（視野 6.5 cm），兩側聲帶韌帶外側各有一「空洞」，係聲帶甲杓肌萎縮，在鬆弛狀態下所形成。選取工具列中的「長度」、「夾角」及「多邊形」等小圖示，測出前聯合（a 線）厚度爲 2.4 mm，右側膜性聲帶直線（b 線）長度爲 16.1 mm，左側膜性聲帶直線（c 線）長度爲 16.4 mm (A)，兩側膜性聲帶直線夾角（弧形虛線）爲 35.8° (B)，而聲門氣道（虛線圈）面積爲 137.7 mm^2 (C)。

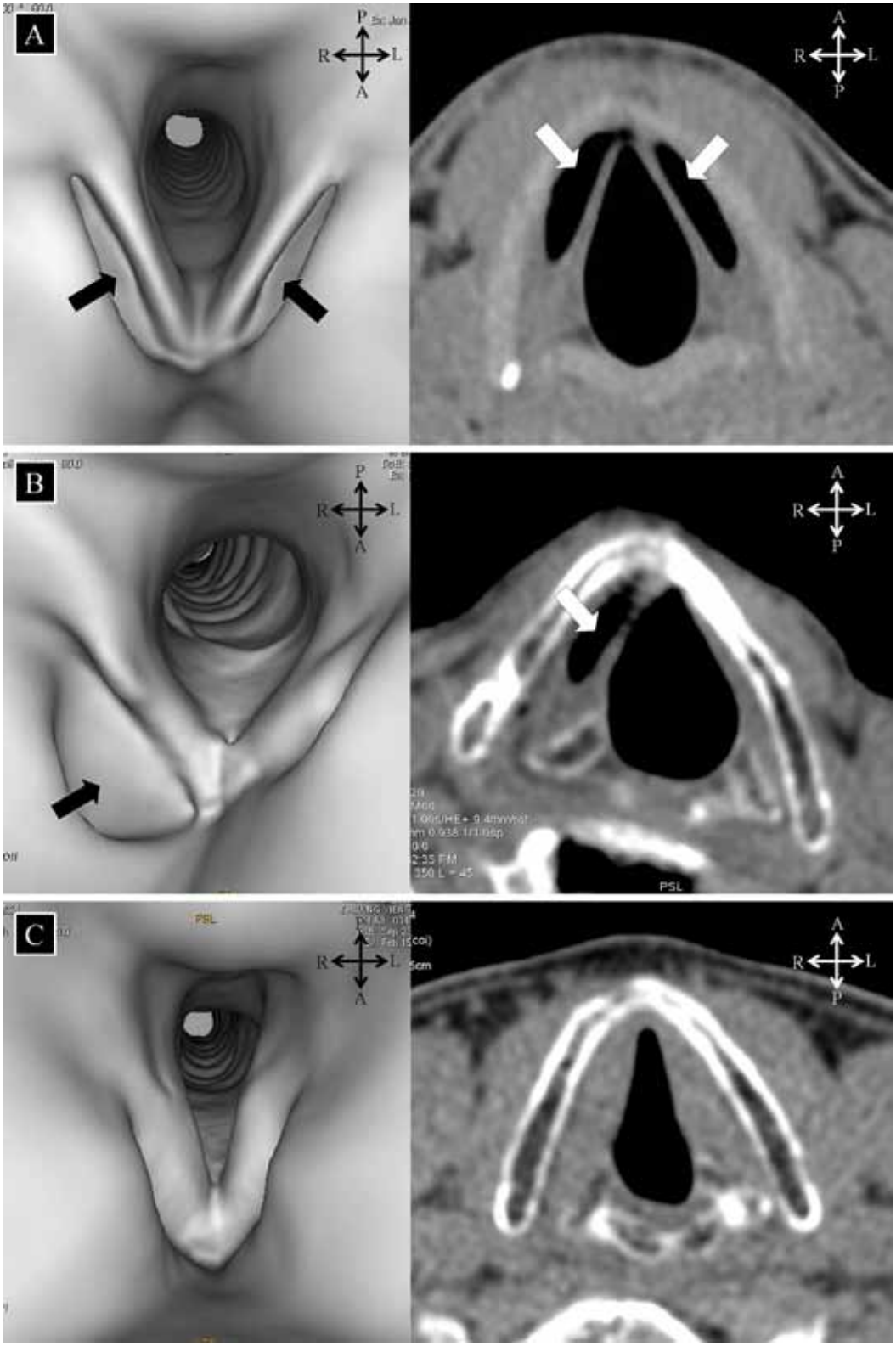


圖 3 A：19 歲女性，虛擬喉鏡見到兩側聲帶甲杓肌有凹陷（實心箭號），軸狀聲門切面顯示兩側聲帶韌帶外側各有一「空洞」（空心箭號），確診爲兩側聲帶萎縮。B：94 歲男性，虛擬喉鏡見到右側聲帶甲杓肌處有凹陷（實心箭號），軸狀聲門切面顯示該側聲帶韌帶外側各「空洞」（空心箭號），故診斷爲右側聲帶萎縮。C：68 歲男性，虛擬喉鏡見到兩側聲帶甲杓肌處無凹陷，軸狀聲門切面顯示兩側聲帶韌帶外側亦無「空洞」，故爲正常聲帶。

統計分析

所有資料都收集於微軟試算表 2000，統計分析也是使用該軟體。比較老年族群、聲帶萎縮與男女性別之人數差異時，使用卡方檢定（X^2 test）。比較各個聲帶參數在老年族群、聲帶萎縮與男女性別間的差異時，使用雙尾 t 檢定（two-tail t-test）。以上檢定之 α 值均設爲 .01。

結　果

共有 17.6%（41 of 233）爲老年人，其中 58.5%（24 of 41）爲女性，57.3%（110 of 192）非老年患者爲男性，但是，老年患者在男女性別間並無差異（p= .0647）。不論男性或女性，陽性者在老年或非老年人間並無差異（p= .8232 & .1337）（表 1）。 10.2%（13 of 127） 的男性與 18.9%（20 of 106）之女性爲陽性者，固然 60.6%（20 of 33）陽性者爲女性，57%（114 of 200）陰性者爲男性，但是，陽性者或陰性者在男女性別間並無差異（p= .0599）。男女陽性者間年齡並無差異（p= .4848），但女性陽性者年齡明顯小於女性陰性者（p= .0002）；此外，男性陰性者年齡明顯小於女性陰性者（p= .0090）（表 2A）。

男性陽性者與女性陽性者之前聯合厚度並無差異（p= .0538），但女性陽性者數值明顯小於女性陰性者（$p=7.66\times10^{-6}$）（表 2B）。在兩側膜性聲帶直線長度比例（右 / 左）方面，不論陽性者或陰性者、男性或女性，數值均無差異（表 2C）。在兩側膜性聲帶平均直線長度方面，不論陽性或陰性，男性數值均明顯大於女性（p= .0003 & 1.92×10^{-21}）；不論男性或女性，陽性者數值均明顯大於陰性者（p= .0097 & .0002）（表 2D）。在兩側膜性聲帶直線夾角方面，男女陽性者間的數值並無差異，女性陰性者之數值明顯大於男性陰性者（p= .0015）；不論男性或女性，陽性者與陰性者間數值並無差異（p= .4311 & .2396）（表 2E）。

在聲門氣道面積方面，不論陽性者或陰性者，男性數值均明顯大於女性（p= .0008 & 5×10^{-19}）；男性陽性者與男性陰性者間數值並無差異（p= .0200），但女性陽性者數值明顯大於女性陰性者（p= .0006）（表 2F）。

表 1　聲帶萎縮在不同性別中老人與非老人之比較

N=233	聲帶萎縮 有（陽性者）（n=33）	聲帶萎縮 無（陰性者）（n=200）	p值
男（n=127）	13	114	
老人	2	15	
非老人	11	99	.8232
女（n=106）	20	86	
老人	2	22	
非老人	18	64	.1337

討　論

目前已有各種方法試圖獲得正常活人聲帶的相關參數，卻各有其限制：（1）喉閃頻內視鏡 [7]：僅能獲得相對長度之「影像參考值」，無法精確地測量其距離；（2）合併雷射投射之高速聲帶圖（laser projection in high-speed glottography）[8]：是一種不錯的方法，但本院沒有該儀器；（3）超音波 [9]：無法測量聲門氣道面積；（4）側面攝片 [10]：無法對尚未發生鈣化之喉部軟骨進行研究，因缺乏不透光之影像標記；（5）在進行喉部直達鏡顯微

表 2　聲帶相關參數之雙尾 t 檢定結果

A.（歲）	陽性者	陰性者	p值
男	39.5±23.4	44.5± 18.3	.4848
女	35.0±15.0	51.5± 18.8	.0002 *
p值	.5630	.0090 *	

B.（mm）	陽性者	陰性者	p值
男	2.57±1.26	3.08±1.62	.2079
女	1.78± .40	2.67±1.51	7.66×10^{-6} *
p值	.0538	.0633	

C.（右/左）	陽性者	陰性者	p值
男	1.00± .04	1.01± .05	.4057
女	1.00± .03	1.02± .05	.0450
p值	.9630	.3998	

D.（mm）	陽性者	陰性者	p值
男	17.10±1.94	15.33±2.40	.0097 *
女	14.05±2.03	11.84±2.15	.0002 *
p值	.0003 *	1.92×10^{-21} *	

E.（°）	陽性者	陰性者	p值
男	40.99±10.71	38.37±10.37	.4311
女	40.48± 9.11	43.37±11.15	.2396
p值	.8920	.0015 *	

F.（mm^2）	陽性者	陰性者	p值
男	205.04±55.76	161.13±48.35	.0200
女	132.91±30.75	102.36±35.17	.0006 *
p值	.0008 *	5×10^{-19} *	

A：年齡；B：前聯合厚度；C：兩側膜性聲帶直線長度比例；D：兩側膜性聲帶平均直線長度；E：兩側膜性聲帶直線夾角；F：聲門氣道面積；* p < .01

手術時直接測量[11]：所呈現的爲全身麻醉及已接受氣管內插管時的聲帶，並非自然呼吸狀態下的狀況；(6) 電腦斷層[12]：需曝露於游離輻射，應用於正常受試者時須考量人體試驗倫理議題；(7) 磁振造影[13,14]：在掃描一開始時就需找到聲門平面，較難事後使用 2 維多層面數位重建技術補救，然而，聲門平面角度及位置因人而異，頗須客制化的操作技術，無異於爲難不諳喉科學之放射技術人員。

電腦斷層之 2 維多層面數位重建（multi-planar reformatting）是一種運用於電腦斷層之影像後處理技術，可以依據判讀者需要製造出不同切面的新影像，技術員只需依循固定模式操作儀器，較不需客制化的技術，若使用薄的切片厚度，在掃描結束後就可組成較佳品質的影像，提供較佳的空間解析度，進而提供較佳品質的表面數位重建或 2 維多層面數位重建，甚至是 3 維立體數位重建（volume rendering）。表面數位重建可以依據判讀者需要製造出「虛擬喉鏡」檢查的效果，除卻傳統喉科檢查對患者所造成的不適，並可製造出特殊的角度或畫面[15,16]；2 維多層面數位重建可以依判讀者需要製造出不同切面的新影像。

很難請正常健康者去接受電腦斷層進行聲帶研究，畢竟需曝露於游離輻射，除非有臨床適應症，於是，那些曾於急診接受頸椎電腦斷層之患者，提供了絕佳的「一般」族群，本文所選個案數（n=233）足以在是否爲老年人、是否具聲帶萎縮與男女性別間不造成差異（表 1）。標準的頸椎電腦斷層所組成連續軸狀切面乃垂直於頸部脊髓正中線，目的在於方便急診科或放射科醫師判讀是否有頸椎骨折，然而，聲門平面不見得恰巧就位於這樣的軸狀切片，畢竟每個人會有不同程度的傾斜或扭轉，因此，必須藉助 2 維多層面數位重建，進而找到恰巧位於兩側杓狀軟骨尖端與前聯合後端的軸狀聲門切面。

由於本文是以滑鼠在電腦螢幕上劃線或劃圈作測量，爲了避免在不同人之間操作方法不同所致之差異，本文全部資料的測量均由第一作者一人爲之。固然同一人對同一目標，以同樣的操作方法進行測量時，依然存在著細微的操作誤差，但由於資料數目龐大，測量者又只有一位，故僅以第一次測量後的結果數據進行分析，這是本文可能的限制。由於大多數未滿 18 歲者之杓骨軟骨並未發生鈣化，在電腦斷層切面下往往與聲帶肌肉不易區別，很難在 2 維多層面數位重建明確地找到其尖端，故排除於本研究。

無論如何，這些影像後處理技術仍無法反應出聲門動態與黏膜眞實狀態[15,16]，無法連結臨床症狀，或與其他評估聲帶萎縮的方式作爲比較或參考依據，故僅能從「聲帶甲杓肌萎縮」去診斷聲帶萎縮，爲此研究之限制。固然一側或兩側聲帶甲杓肌處有「凹陷」即可認定是聲帶萎縮[1]，但目前尚無客觀之定義「凹陷」的程度，本文暫且定性爲需足以在「兩側杓骨尖端與前聯合最後端」3 點所構成之軸狀聲門切面上，造成至少一側聲帶韌帶外側有「空洞」（圖 3A&B），否則僅能算是正常聲帶（圖 3C）。本文發現，在一般呼吸狀態下，鬆弛的膜性聲帶並非呈現直線，而是呈現圓弧形（圖 2），故僅能就杓狀軟骨尖端至前聯合後端之直線距離，定義爲膜性聲帶「直線」長度。兩側膜性聲帶直線長度比例大約爲 1.0，在具聲帶萎縮與否、男女性別間之數值均無差異（表 2C）。

固然聲帶萎縮可以出現在年輕族群，但普遍認爲與老化有關，在老年人應該較多，然而，本文卻發現，不論男性或女性，有無聲帶萎縮者在老年或非老年人間並無差異（表 1），最令人驚訝的是，在女性，具聲帶萎縮者之年齡居然較不具聲帶萎縮者年輕（表 2A）。聲帶萎縮可能會合併喉部相關結構的變化，對女性的影響較男性明顯；在前者，前聯合厚度較小（表 2B），兩側聲帶平均直線長度較長（表 2D），聲門氣道面積較大（表 2F）；在後者，僅兩側聲帶平均直線長度較長（表 2D）。

結　論

本文僅能從「聲帶甲杓肌萎縮」去診斷聲帶萎縮，共 14.2%（33 of 233）爲「陽性者」，其中僅 12.1%（4 of 33）爲老年人。不論男性或女性，有無聲帶萎縮者在老年或非老年人間並無差異；在女性，具聲帶萎縮者之年齡居然較不具聲帶萎縮者年輕。因此，聲帶萎縮並非在老年人較多，或許僅是一種解剖學上的異常，不全然是老化所致。

參考文獻

1. 陳建志、熊明旺、林曜祥、王智弘：以微粉化AlloDerm注射喉整形術治療聲帶萎縮。台耳醫誌 2008;43:121-8.
2. Takano S, Kimura M, Nito T, Imagawa H, Sakakibara K, Tayama N: Clinical analysis of presbylarynx--vocal fold atrophy in elderly individuals. Auris Nasus Larynx 2010;37:461-4.
3. Baker K, Ramig L, Luschi E, Smith M: Thryoartenoid muscle activity associated with hypophonia in Parkinson Disease and aging. Neurology 1998;51:1592-8.
4. Luschei E, Ramig L, Baker K, Smith M: Discharge characteristics of laryngeal single motor units during phonation in young and older adults and in persons with Parkinson Disease. J Neurophysiology 1999;81:2131-9.
5. 陳建志：喉部病灶在胸部X光及聽診正常之慢性咳嗽所扮演的角色。慈濟醫學 2007;19:145-51。
6. 李家和、江豐裕、郭文烈、阮愷輝：高解析度喉纖維鏡用於閃頻錄影檢查。中耳醫誌 1994;29:497-503。
7. 黃澤人，Jiang JJ：聲門面積動態變化的測量。中耳醫誌 2001;36:141-7。
8. Schuster M, Lohscheller J, Kummer P, Eysholdt U, Hoppe U: Laser projection in high-speed glottography for

high-precision measurements of laryngeal dimensions and dynamics. Eur Arch Otorhinolaryngol 2005;262:477-81.
9. Hu Q, Zhu SY, Luo F, Gao Y, Yang XY: High-frequency sonographic measurements of true and false vocal cords. J Ultrasound Med 2010;29:1023-30.
10. Roers F, Mürbe D, Sundberg J: Predicted singers' vocal fold lengths and voice classification-a study of x-ray morphological measures. J Voice 2009;23:408-13.
11. Su MC, Yeh TH, Tan CT, Lin CD, Linne OC, Lee SY: Measurement of adult vocal fold length. J Laryngol Otol 2002;116: 447-9.
12. Kallmes DF, Phillips CD: The normal anterior commissure of the glottis. AJR Am J Roentgenol 1997;168:1317-9.
13. Flaherty RF, Seltzer S, Campbell T, Weisskoff RM, Gilbert RJ: Dynamic magnetic resonance imaging of vocal cord closure during deglutition. Gastroenterology 1995;109:843-9.
14. Cüreoğlu S, Osma U, Ozates M: Normal configuration of the anterior commissure of the glottis on magnetic resonance imaging. Eur Arch Otorhinolaryngol 2000;257:219-20.
15. Byrne AT, Walshe P, McShane D, Hamilton S: Virtual laryngoscopy--preliminary experience. Eur J Radiol 2005;56: 38-42.
16. Yan Y, Luo S, McWhorter A: Virtual laryngoscopy: a noninvasive tool for the assessment of laryngeal tumor extent. Laryngoscope 2007;117:1026-30.

The Prevalence of Vocal Fold Atrophy in Patients Undergoing Emergent Cervical Spinal Computed Tomography

Jiann-Jy Chen[1,2,3,*], Li-Kuo Shen[3], Hsing-Won Wang[4,5], Dem-Lion Chen[6], Yao-Mao Tang[7]

[1] *Department of Neurology, China Medical University Hospital, Taichung, Taiwan*
[2] *China Medical University, Taichung, Taiwan*
[3] *Department of Medical Imaging,* [4] *Otolaryngology, Taipei Medical University & Shuang Ho Hospital, New Taipei, Taiwan*
[5] *Taipei Medical University - Graduate Institute of Clinical Medicine, Taipei, Taiwan*
[6] *G-Home Otorhinolaryngologic Clinic, Kaohsiung, Taiwan*
[7] *Tang Yao Mao Otolaryngologic Clinic, Changhua, Taiwan*

Received: May. 7, 2012; Accepted: July. 24, 2013

Abstract

Background and Purpose: Vocal fold atrophy (VFA) is thought to be associated with aging and prevalent in the aged population. However, current studies have been limited to otolaryngological outpatients alone. We performed a retrospective imaging study to investigate the general prevalence of VFA in patients undergoing emergent cervical spinal computed tomography.
Methods: In total, 233 patients, including 127 males and 106 females (age, 46.0 ± 19.2 years), who underwent emergent cervical spinal computed tomography between September 2010 and April 2011, were enrolled. Virtual laryngoscopy with surface rendering was performed to differentiate VFA from other conditions, and 2D multiplanar reformatting was performed to generate the axial cut at the vocal points of the bilateral arytenoid cartilages and the posterior end of the anterior commissure. The anterior commissure thickness, the straight length and included angle of the bilateral membranous vocal folds, and the glottic airway area were measured. A chi-square test and a two-tailed t test ($\alpha = 0.01$) were used for statistical analysis of the data.
Results: A total of 14.2% (n = 33) patients had VFA; of these, 12.1% (4/33) were aged ≥65 years. No statistical difference was observed in VFA between the aged and nonaged in the male or female patients ($p = 0.8232$ and 0.1337, respectively). However, the influence of VFA was more prominent in the females than the males; the females with VFA revealed thinner anterior commissures ($p = 7.66 \times 10^{-6}$), longer straight lengths of bilateral membranous vocal folds ($p = 0.0002$), and larger glottic airways ($p = 0.0006$) compared with those in females without VFA; on the other hand, males with VFA revealed longer straight lengths of bilateral membranous vocal folds ($p = 0.0097$) compared with those in males without VFA.
Conclusions: VFA was not more prevalent in the aged than in the common adult population, but it may be an anatomical variation that is not caused by aging.

Key words: vocal fold atrophy, virtual laryngoscopy, surface rendering, 2D multiplanar reformatting, cervical spinal computed tomography

*Correspondence to: Dr. Jiann-Jy Chen, Department of Neurology, China Medical University Hospital, No.2, Yude Road., North District., Taichung City 40447, Taiwan (R.O.C.)

Tungs' Medical Journal 7 (2013) 26-30

Case Report

A Right Humeral Pathologic Fracture due to Metastatic Renal Cell Carcinoma at 12 Years Post Right Nephrectomy: A Case Report and Literature Review

Zhon-Min Huang[1,*], Jue-Hawn Yin[1], Yu-Chieh Cheng[2], Min-Che Tung[1]

[1]Divisions of Urology, Department of Surgery, [2]Department of Orthopedics, Tungs' Taichung Metro Harbor Hospital, Taichung, Taiwan

Received: Sep. 28, 2011; Accepted: Jun. 17, 2013

Abstract

The skeleton is the second most common site of distant metastasis from renal cell carcinoma. Although radiotherapy and chemotherapy are ineffective in most patients with RCC, surgery is a safe and reliable modality to achieve local tumor control, restore mechanical bone stability, relieve pain, and increase survival. A longer interval between diagnosis of renal cancer and that of osseous metastasis and the absence of extraosseous metastases are predictive of higher survival. Here, we report a case of a 77-year-old male who suffered from a right humeral pathologic fracture caused by metastatic renal cell carcinoma at 12 years post right nephrectomy. He underwent wide excision for the right humeral tumor and had an uneventful 6-month follow-up period.

Key words: kidney, renal cell carcinoma, bone metastasis

Introduction

Renal cell carcinoma (RCC) is the most common renal tumor and is characterized by a lack of early warning signs. As a result, one-third of patients have been found to have metastatic disease at the initial presentation[1]. RCC metastasizes via lymphatic or venous routes, and the most common site for metastasis of RCC is the lung, followed by the bone, liver, and brain[2,3]. Surgical excision is a safe and reliable method for local tumor control, pain relief, and providing a good functional outcome[4,5]. Here, we report a patient with bone metastasis from RCC who underwent wide excision of a right humeral tumor.

Case Report

A 77-year-old man underwent a right nephrectomy for a localized RCC 12 years previously. He had not received follow-up since 1 year postoperatively. He suffered from disability and local tenderness with a limited range of motion over his right shoulder for approximately 3 months. Ecchymosis over the right upper arm, right shoulder, and right upper chest was observed (Fig. 1). The radiographic findings revealed a right proximal humeral fracture (Fig. 2). A computerized tomography (CT)-guided biopsy of the right humerus revealed metastatic RCC. An abdominal CT scan demonstrated status post right nephrectomy, normal left kidney, and no lymphadenopathies (Fig. 3). A whole-body bone scan revealed a focal area of increased uptake of radioactivity involving the fractured right humerus that corresponded to the pathological fracture. A chest radiograph revealed no active lung lesion. He underwent wide excision for the right humeral tumor and open reduction and internal fixation (ORIF) using a Rush pin and cement augmentation (Fig. 4). Pathological examination of the excised bone confirmed metastatic RCC (Fig. 5). The patient's postoperative course was uneventful.

*Correspondence to: Zhon-Min Huang, Divisions of Urology, Department of Surgery, Tungs' Taichung MetroHarbor Hospital. No. 699, Sec. 8, Taiwan Blvd., Wuqi Dist., Taichung City 43503, Taiwan (R.O.C.)

During the 6-month follow-up period, the function of the patient's right arm was restored. No local tumor recurrence or distant metastasis was found.

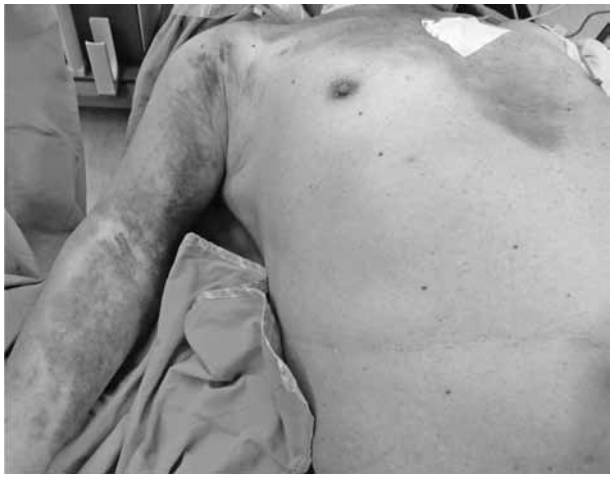


Fig. 1 Ecchymosis over the right arm and upper chest.

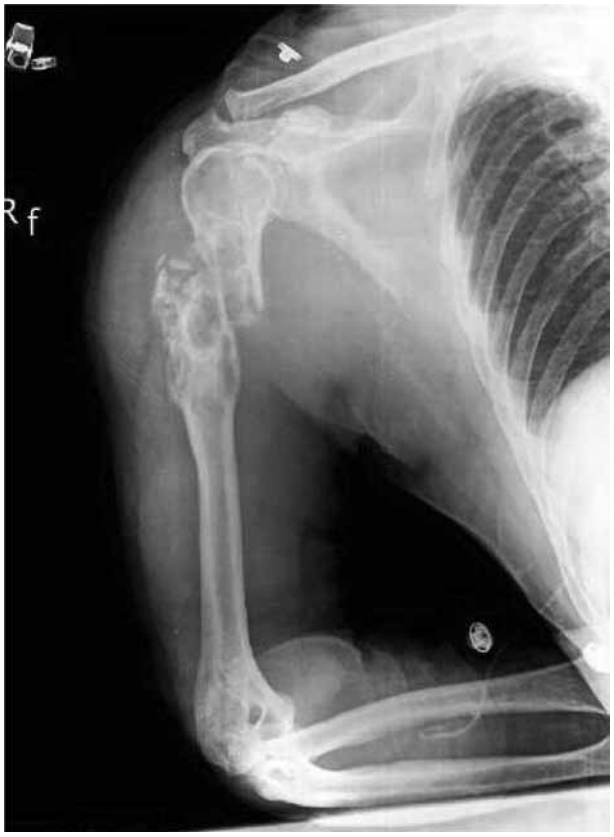


Fig. 2 An X-ray image showing the right humeral pathologic fracture.

Discussion

RCC is the most common malignancy arising from the kidney, and distant metastasis has been found in 30%-60% of these patients at initial presentation. The most common site for metastasis of RCC is the lung, followed by the skeleton, liver, and brain. Bone lesions in RCC are predominantly osteolytic[6], and the reported incidence of skeletal metastases

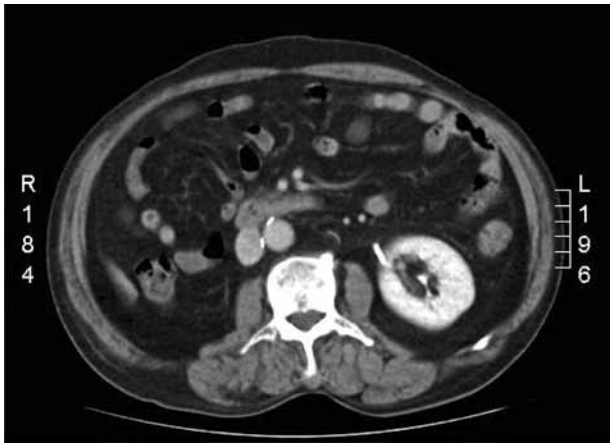


Fig. 3 A CT scan showing that the patient underwent a right nephrectomy and normal left kidney.

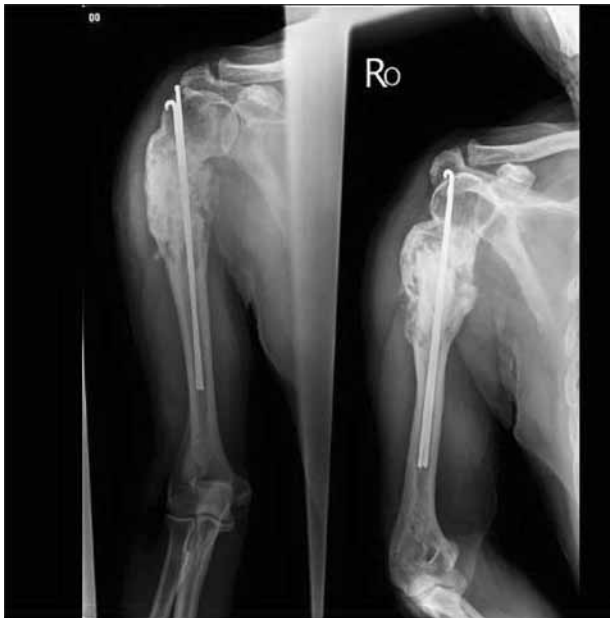


Fig. 4 An X-ray image demonstrating the pathologic fracture of the right proximal humeral shaft post excision of the bone tumor, bone cement implantation, and intramedullary pin fixation; with a stable union.

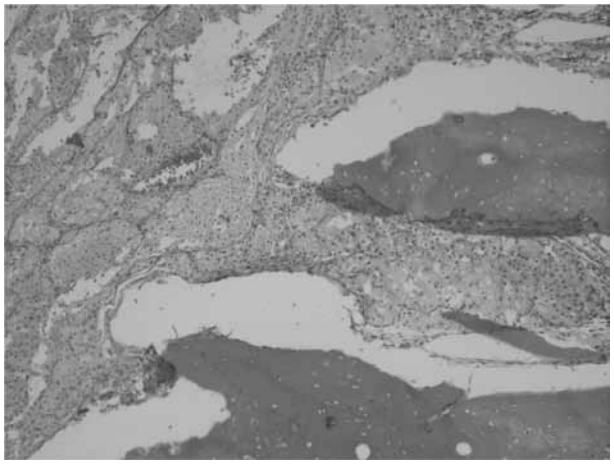


Fig. 5 Pathological staining of tumor cell nests within a fine vascular network with bone destruction (H & E staining; magnification, X100).

from RCC is 17%-50%[7].

Approximately half of patients presenting with metastases die within the first year, and only 10% survive for >5 years[8]. The 5-year-survival rate of patients with a solitary bone metastasis from RCC is between 35% and 60%[9]. Althausen et al. reported that presentation without metastases, a long disease-free interval between nephrectomy and the first metastasis, appendicular skeletal location, and solitary metastases were associated with longer survival. Fottner et al. demonstrated that age <65 years, absence of pathological fractures, and tumor-free resection margins were predictive of higher survival. Gender et al. reported that the metastatic location, time between diagnosis of RCC and treatment of metastatic disease, incidence of local recurrence, and radiotherapy and chemotherapy did not influence survival[10]. Jung et al. concluded that the presence of a single osseous metastasis, wide lesion resection, and a history of nephrectomy were independent predictors of survival in patients with RCC. Yutaka et al. demonstrated that patients in whom bone metastasis developed within 24 months or longer after the diagnosis of RCC or extraosseous metastases did not develop had significantly longer survival[11]. Although our patient was older, a solitary appendicular bone metastasis from RCC and the long interval between bone metastasis and nephrectomy implied a favorable prognosis.

Regarding bone metastases from RCC, administration of the bisphosphonate zoledronic acid has demonstrated the ability to significantly delay or prevent skeletal complications[12]. The main treatment options for patients with advanced RCC include interleukin-2 and interferon-alpha, but they have somewhat limited efficacy in this disease. The targeted therapy era has revolutionized the systemic approach to the treatment of advanced RCC[13].

Indications for surgical intervention are intractable pain, impending or present pathological fracture, and solitary bone metastasis. Smith et al. reported that most patients with RCC survive long enough to benefit from a palliative orthopedic procedure[14]. Grant et al. recommended palliative orthopedic intervention in any patient with a life expectancy of >2 months[15]. Surgical intervention is a safe and reliable treatment for local control of metastatic RCC in the bone, pain relief, restoring mechanical bone stability, and enabling restoration of good function. Our patient underwent wide excision for the right humeral tumor and ORIF using a Rush pin and cement augmentation for his pathologic fracture due to metastatic RCC. The bone stability and function were restored following the surgical procedures.

Here, we report the case of a 77-year-old male with a right humeral pathological fracture caused by metastatic RCC at 12 years post right nephrectomy. He underwent wide excision for the right humeral tumor and had an uneventful 6-month follow-up period.

References

1. Mani S, Todd MB, Katz K, Poo WL. Prognostic factors for survival in patients with metastatic renal cancer treated with biological response modifiers. J Urol 1995; 154: 35-40.
2. Motzer RJ, Bander, NH, Nanus DM. Renal-cell carcinoma. N Engl J Med 1996; 335: 865-75.
3. Russo P, Renal cell carcinoma: presentation, staging, and surgical treatment. Semin Oncol 2000; 27: 160-76.
4. Yehuda K, Jacob B, William MP, Kristen LK, Juza C, Ofer M, et al. Metastatic renal cell carcinoma of bone: indications and technique of surgical intervention. J Urol 2000; 164: 1505-8.
5. Jacob B, Ofer M. Bone matastases of renal cell carcinoma: the role of surgery. IMAJ 2002; 4: 376-8.
6. Adiga GU, Dutcher JP, Larkin M, Garl S, Koo J. Characterization of bone metastases in patients with renal cell cancer. BJU Int 2004; 93: 1237-40.
7. Althausen P, Althausen S, Jennings LC, Mankin HJ. Prognostic factors and surgical treatment of osseous metastases secondary to renal cell carcinoma. Cancer 1997; 80: 1103-9.
8. Motzer RJ, Bacik J, Mazumder M. Prognostic factors for survival of patients with stage IV renal cell carcinoma: memorial sloan-kettering cancer center experience. Clin Cancer Res 2004; 15: 6202S-3S.

9. Jung ST, Ghert MA, Harelson JM, Scully SP. Treatment of osseous metastases in patients with renal cell carcinoma. Clin Orthop Relat Res 2003; 409: 223-31.
10. Andreas F, Melinda S, Lilly W, Michael S, Andrea BM, Volkmar J, et al. Bone metastases from renal cell carcinoma: patient survival after surgical treatment. BMC Musculoskel Dis 2010; 11: 145-50.
11. Yatuka T, Nobuo S, Toru H, Takashige A, Tomoshige A, Ataru S, et al. Survival and prognostic classification of patients with metastatic renal cell carcinoma of bone. Eur Urol 2007; 52: 163-9.
12. Lipton A, Colombo-Berra A, Bukowski RM, Rosen L, Zheng M, Urbanowitz G. Review skeletal complications in patients with bone metastases from renal cell carcinoma and therapeutic benefits of zoledronic acid. Clin Cancer Res. 2004; 10: 6397-6403.
13. Chakshu S, Jennifer J, Mark C, Anthony M. Renal cell carcinoma bone metastases: clinical advances. Ther Adv Med Oncol 2010; 2: 75-83.
14. Smith EM, Kursh ED, Makley J, Resnick MI. Treatment of osseous metastases secondary to renal cell carcinoma. J Urol 1992; 148: 784-7.
15. Grant TT, dekernion JB, Treatment of skeletal metastases from urologic malignancies. Urology 1978; 11: 563-7.

右側腎細胞癌於腎臟切除手術十二年後轉移造成右側肱骨病理性骨折病例報告及文獻探討

黃鐘銘[1,*] 殷約翰[1] 鄭宇傑[2] 童敏哲[1]

童綜合醫院 外科部 [1]泌尿外科 [2]骨科部

受文日期：民國 100 年 9 月 28 日；接受刊載：民國 102 年 6 月 17 日

摘要

骨頭是為腎細胞癌第二常見的轉移部位。放射線治療及化學治療對大部分腎細胞癌的病人都不是那麼有效。手術治療是一個安全而且可以達到局部腫瘤控制、保存骨頭穩定、減緩疼痛及增加存活率的治療方式。骨頭轉移離腎細胞癌診斷出來的時間越久以及沒有骨頭以外的轉移對預後越好。我們報告一個 77 歲的男性，他因為腎細胞癌於十二年前切除右側腎臟，這次因為轉移造成右側肱骨病理性骨折。他接受了手術治療，手術後經過六個月的追蹤，恢復良好，也沒有復發或轉移的現象。

關鍵詞：腎臟、腎細胞癌、骨頭轉移

* 通訊作者：黃鐘銘 童綜合醫療社團法人童綜合醫院 外科部 泌尿外科 43503 臺中市梧棲區臺灣大道八段 699 號

Case Report

單純以轉移性淋巴腮腺腫瘤為表現的默克爾細胞癌

張紘頤[1] 蔡青劭[1,2] 曹唐義[3] 李佳茹[2] 葉啟源[4] 黎瓊柱[1,*]

童綜合醫療社團法人童綜合醫院 耳鼻喉科[1] 醫研部[2] 病理部[3] 放射腫瘤科[4]

受文日期：民國 102 年 1 月 31 日；接受刊載：民國 102 年 3 月 15 日

摘要

默克爾細胞癌是一種極少見的皮膚未分化癌，它來自於表皮中一種神經內分泌細胞默克爾細胞演變而來，伴有高度侵略性，多發生在長時間接受日照的白年老男性，常發現在頭頸部，但仍有少數人，單以淋巴轉移而找不出原發性部位，因數據與案例不多，還沒有一個明確的確切治療定論。本篇報告一位 79 歲男性病患因直徑約三公分的輕微壓痛腮腺腫塊來就診，於耳鼻喉科行淺表腮腺切除術送病理化驗後診斷出是轉移性默克爾細胞癌，在經過一連串腫瘤勘查甚至接受全身正子掃描後，仍找不出原發部位。術後繼續於本院接受頸部淋巴區域的放射線治療。現追蹤長達半年，無任何術後併發症與復發現象。現持續於本院門診追蹤。本文討論少見的原發部位不明之轉移性默克爾細胞癌，並單以唾液腺淋巴腫塊來表現，此案例希望提供醫師參考。

關鍵詞：默克爾細胞癌、唾液腺腫瘤

前 言

默克爾細胞癌（Merkel cell carcinoma）於 1972 年由 Toker 等人首次提出，爲一種罕見且惡性的皮膚腫瘤，屬於神經內分泌細胞所起源的腫瘤，約有 46.3% 至 53% 發現於頭頸部 [1-3]。根據 2009 年流行病學和最終結果計畫數據庫審查（Surveillance Epidemiology and End Results；SEER），分析 1973 年至 2006 年，共 3804 位默克爾細胞癌的病患，列出十個最常見的部位，其中約 91.8% 的人病灶出現在皮膚，然而找不出原發部位的仍占約 0.8%[4]。默克爾細胞癌其存活率與原發腫瘤的大小有關，腫瘤較大其預後較差，淋巴轉移其存活率也比無淋巴轉移差，無淋巴轉移其十年存活率約七成，而有淋巴轉移十年存活率剩不到五成，找不出原發性病灶的人十年存活率約爲四成，而遠端轉移十年存活率僅 2 成 [4]。在國內關於的默克爾細胞癌的相關文獻並不多，其中找不出原發部位的腫瘤更是少之又少，特提出報告並加以討論。

* 通訊作者：黎瓊柱醫師 童綜合醫療社團法人童綜合醫院 耳鼻喉科 43503 臺中市梧棲區臺灣大道八段 699 號

病例報告

一位 79 歲男性病患，曾有第三期前列腺癌（cT3N0M0）病史，已接受過完整的放射治療，並且定期於本院泌尿科追蹤。另外，尚有肺結核病史，唯現已痊癒。病人在 2012 年 7 月因右耳下腫塊持續兩個禮拜而來本院耳鼻喉科就診，此腫塊並無壓痛且持續變大。病人無酗酒、嚼食檳榔與吸煙的習慣，最近也無體重減輕、張口困難、吞嚥困難、吞嚥疼痛、發燒、寒顫和盜汗等現象。

在臨床檢查發現此腫塊爲單側，直徑約 3 公分。病人無甲狀腺腫大現象，其他頭頸部檢查，包括纖維鼻竇咽喉內視鏡也無異常發現，而十二對腦神經也完整無陽性發現，右側耳上三公分處有一個類似老人斑的病灶。

電腦斷層掃描結果顯示一個約 2.7 × 1.5 × 1.7 公分大小包覆完整顯影之低密度的腫瘤（圖 1），位置約在右側腮腺尾端，其前內側與胸鎖乳突肌接觸。胸部 X 光檢查則爲以前肺結核留下來的陳舊性病灶。

經解釋後病人接受表淺腮腺切除手術（superficial parotidectomy），腫瘤完整切除，爲一個帶有淡褐色略堅硬的囊狀腫瘤大小約 3 公分（圖 2），耳大神經穿越其中，術中完整保留也無傷到顏面神經。

免疫組織化學染色報告 synoatophsyin、NSE、CD56 和 chromagranin-A 爲陽性反應，可看出是一種神經內分泌細胞所變異而來；CD45 爲陰性，CK、CD20、和 Ki-67 爲陽性反應（圖 3），經診斷爲 3 顆淋巴組成之轉移性的默克爾細胞癌。因原發部位不明，加上病患五年內曾罹患前列腺癌，故會診泌尿科排除前列腺癌的復發，經抽血檢查，腫瘤指標數據都正常，故泌尿科醫師認爲復發機率不高。會診皮膚科詳細檢查有無其他可疑皮膚病灶，皮膚科醫師分別指出耳上的老人斑與肛門黏膜的小潰瘍爲唯二可疑病灶，故安排切片，結果都爲良性。在無明顯原發部位的情況下，安排全身正子掃描，仍無陽性反應。臨床分級爲 cTxN1bM0，第三期，根據 National Comprehensive Cancer Network（NCCN）臨床指引於手術後在頭頸部接受放射線治療 180 cGyx，34 次，總劑量爲 6120 cGy，療程爲 48 天，範圍包括右側頸部淋巴結 II、III、IV 和 V。

病人於術後追蹤至今超過半年，無任何手術併發症或復發現象。因照射部位位於腮腺，所以有口乾現象，其他身體狀況正常（圖 4），追蹤頸部電腦斷層也無異常。

討　論

默克爾細胞癌是一種罕見的高度惡性的神經內分泌腫瘤，主要位於太陽曝露的皮膚部位，好發於 60 至 85 歲男性白人 [4,5]。此病患職業爲農夫，長期曝露於太陽下，可能是罹患此病的危險因子，患者雖然五年內因前列腺癌接受放射線治療，但部位在骨盆腔，而非頭頸部，所以非罹病之危險因子。臨床症狀可以 AEIOU 來診斷，A=Asymptomatic（無症狀性），E=Expanding rapidly（成長快速），I=Immune suppressed（免疫功能受限），O=Older than 50 years（大於 50 歲的老人），U=UV-exposed skin（常接受紫外線曝照），佔三項以上約爲 89%，五項都有只占 7%[6]。

默克爾細胞癌的診斷主要是利用免疫組織化學染色，必須與原始神經外胚層細胞瘤（periphery primitive neuroectodermal tumor）（CK20-、TTF-1-、CD99+）；肺小細胞癌（small cell carcinoma lung）（CK7+、CK20-、TTF-1+、CD99-）；小細胞黑色素瘤（small cell melanoma）（CK20-、NSE+、S-100+）及淋巴瘤（lymphoma）（CD45(-)、CK20-）作區別，默克爾細胞癌其重要免疫組織化學染色爲 CD45(-)、CK(+)、CD20(+)、Ki-67(+)[7-9]。通常病灶大多小於 2 公分，平均爲 0.1 至 0.19 公分，但仍有報告爲 12 至 15 公分 [3,6]。腫瘤可能是肉色，紅色，紫紅色或深紫紅色，可有一個覆微血管擴張的閃亮表面 [7]，它可以迅速擴大規模，甚至可能會潰爛。

根據 NCCN 臨床指引，局部發生的默克爾細胞癌建議手術廣泛性切除並接受術後的放射線治療（50cGy-60cGy），而輔助性的化學治療原則上是用 cisplatin+/-etoposide、carboplatin+/-etoposide、topotecan、cyclophosphamide、doxorubicin（或 epirubicin）或 vincristine（CVA），不過僅適用在擴散出去的病人。Medina-Franco 等人 [10] 曾於 1024 例病患中發現接受過術後放射治療遠比無接受放射治療的復發率低。約

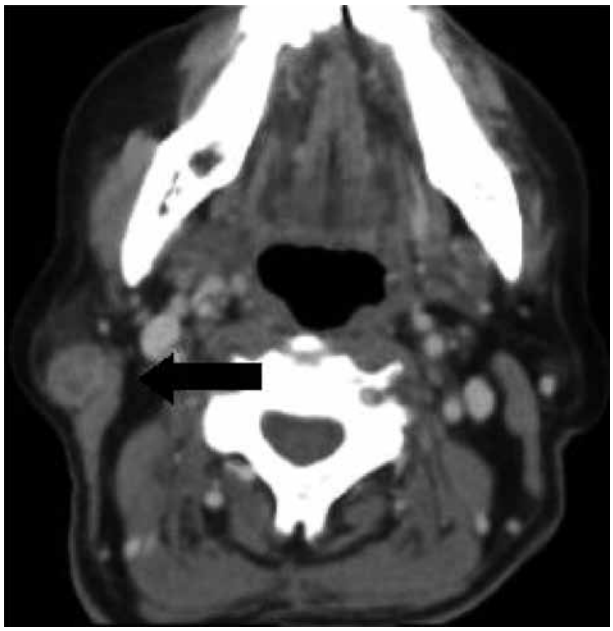


圖 1　右側腮腺一個約 2.7 × 1.5 × 1.7 公分大小包覆完整顯影之低密度的病灶（黑色箭頭處）。

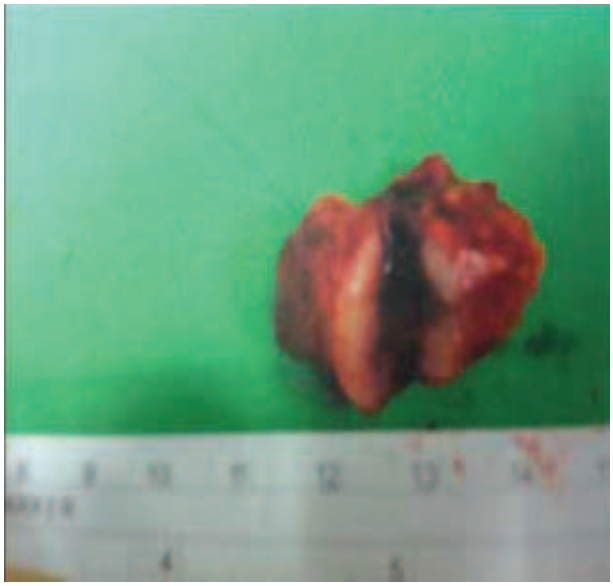


圖 2　爲一結實的囊狀腫瘤約 3 公分大小。

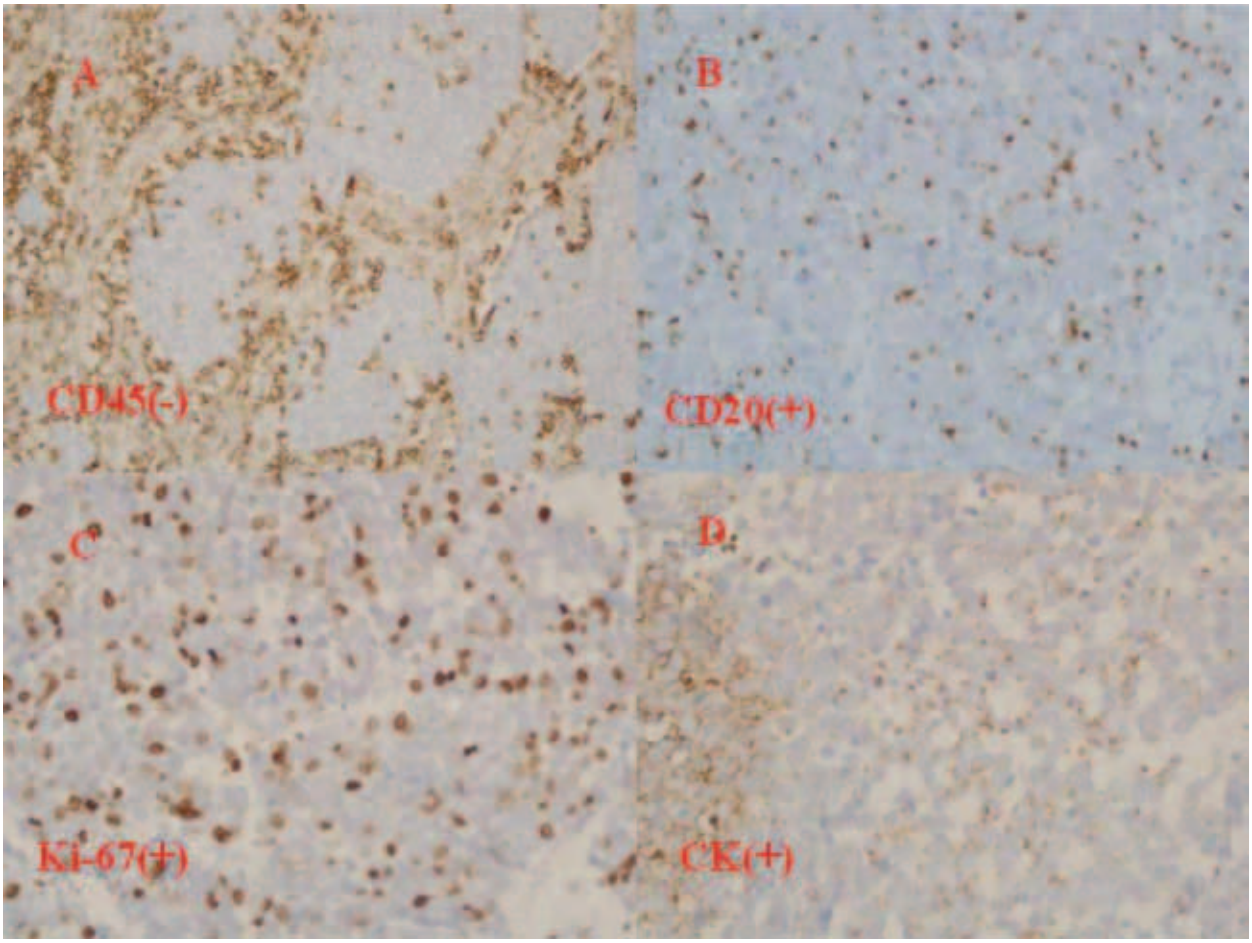
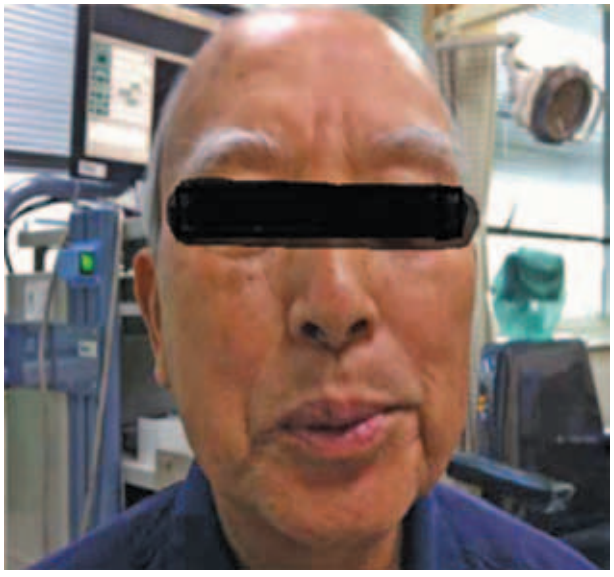


圖 3　免疫組織化學染色：A. CD45 呈陰性反應，（IHC 染色，100×），B. CD20 呈陽性反應，（IHC 染色，100×），C. Ki67 呈陽性反應，（IHC 染色，100×），D. CK 呈陽性反應，（IHC 染色，400×）。



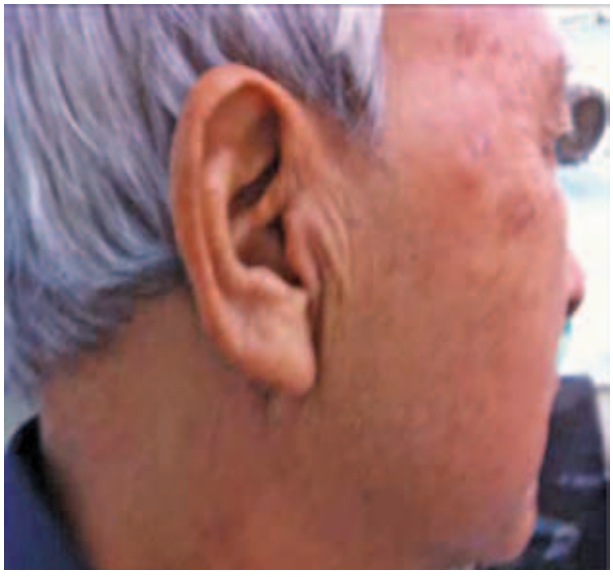


圖 4　病人術後，狀況良好。

70-80% 的病人能在局部開始有病灶時被發現，但因其可在皮膚之上或之下發生，故仍有近 30% 的病人一發現已有淋巴轉移（如同本病例），而有 1-4% 的病人發現已遠端轉移 [11]。其復發機率高，在接受過初次切除的病人，兩年內會有高達四成以上的病患會局部復發 [12]，而以後疾病會淋巴轉移的也高達將近七成，而演變成遠端轉移的更是出現在許多案例 [10]。

此案例爲本院第一個原發部位不明之默克爾細胞癌直接轉移至腮腺淋巴結所呈現的案例，故對腮腺腫塊的病人，必須做完整的頭頸部檢查，纖維鼻竇咽喉內視鏡，影像學檢查，以及細針穿刺或是活體切片，以達成正確診斷，給予病患有效治療，以提高惡性腫瘤的存活率。

參考文獻

1. Tarantola TI, Vallow LA, Halyard MY, Weenig RH, Warschaw KE, Grotz TE, et al. Prognostic factors in Merkel cell carcinoma: Analysis of 240 cases. J Am Acad Dermatol. 2012 Nov 27. pii: S0190-9622(12)01041-9. doi: 10.1016/j.jaad.2012.09.036.
2. Güler-Nizam E, Leiter U, Metzler G, Breuninger H, Garbe C, Eigentler TK. Clinical course and prognostic factors of Merkel cell carcinoma of the skin. Br J Dermatol 2009; 161: 90-94.
3. Kukko H, Böhling T, Koljonen V, Tukiainen E, Haglund C, Pokhrel A, et al. Merkel cell carcinoma - a population-based epidemiological study in Finland with a clinical series of 181 cases. Eur J Cancer 2012; 8: 737-742.
4. Albores-Saavedra J, Batich K, Chable-Montero F, Sagy N, Schwartz AM, Henson DE. Merkel cell carcinoma demographics, morphology, and survival based on 3870 cases: a population based study. J Cutan Pathol 2010; 37: 20-27.
5. Reichgelt BA, Visser O. Epidemiology and survival of Merkel cell carcinoma in the Netherlands. A population-based study of 808 cases in 1993-2007. Eur J Cancer 2011; 47: 579-585.
6. Heath M, Jaimes N, Lemos B, Mostaghimi A, Wang LC, Peñas PF, Nghiem P. Clinical characteristics of Merkel cell carcinoma at diagnosis in 195 patients. J Am Acad Dermatol 2008; 58: 375-381.
7. Goessling W, McKee PH, Mayer RJ. Merkel Cell Carcinoma. J Clin Oncol 2002; 20: 588-598.
8. Buresh CJ, Oliai BR, Miller RT.. Reactivity with TdT in Merkel cell carcinoma: a potential diagnostic pitfall. Am J Clin Pathol. 2008; 129: 894-8.
9. Jensen K, Kohler S, Rouse RV. Cytokeratin staining in Merkel cell carcinoma: an immunohistochemical study of cytokeratins 5/6, 7, 17, and 20. Appl Immunohistochem Mol Morphol. 2000; 8: 310-5.
10. Medina-Franco H, Urist MM, Fiveash J, Heslin MJ, Bland KI, Beenken SW. Multimodality treatment of Merkel cell carcinoma: case series and literature review of 1024 cases. Ann Surg Oncol 2001; 8: 204-208.
11. Ferringer T, Rogers HC, Metcalf JS. Merkel cell carcinoma in situ. J Cutan Pathol 2005; 32: 162-165.
12. Allen PJ, Bowne WB, Jaques DP, Brennan MF, Busam K, Coit DG. Merkel cell carcinoma: prognosis and treatment of patients from a single institution. J Clin Oncol 2005; 23: 2300-2309.

Metastatic Merkel cell carcinoma in the parotid gland with unknown primary site

Hung-Min Chang[1], Stella Chin-Shaw Tsai[1,2], Tang-Yi Tsao[3], Chia-Ru Li[2], Chi-Yuan Yeh[4], Chiung-Chon Li[1,*]

[1]Department of Otolaryngology, [2]Department of Medical Research, [3]Division of Pathology, [4]Department of Radiation Oncology, Tungs' Taichung MetroHarbor Hospital

Received: Jan. 31, 2013; Accepted: Mar. 15, 2013

Abstract

Merkel cell carcinoma is a rare undifferentiated skin carcinoma originating from neuroendocrine cells in the epidermis. Merkel cell is a highly aggressive form of cancer, often occurring in the head and neck area of white elderly men with a history of prolonged sun exposure. Lymph node metastasis is common. However, the primary site of Merkel cell carcinoma is not always identifiable. The management of Merkel cell carcinoma has not been clearly defined because of limited data and only few case reports.

Here, we report the case of a 79-year-old male patient with a mildly tender parotid mass about 3 cm in diameter. Superficial parotidectomy was performed. Pathological analysis indicated metastatic Merkel cell carcinoma. After a series of tumor surveys and whole-body positron emission tomography, the original cancer site remained unknown. Postoperative irradiation of the neck area was performed. During 6-month followup, no postoperative complications or recurrence were observed. In this article, we discuss a rare metastatic Merkel cell carcinoma presenting as a parotid mass with unknown primary site.

Key word: Merkel cell carcinoma, parotid tumor.

*Correspondence to: Dr. Chiung-Chon Li, Department of Otolaryngology, Tungs' Taichung MetroHarbor Hospital, 699, Sec. 8, Taiwan Boulevard, Wuchi, Taichung, 43503, Taiwan (R.O.C.)

Instruction to contributors

This journal provides a forum for medical related articles, such as original researches, clinical analysis, case reports and special review articles, special articles, communication (includes brief communication), images in clinical medicine, editorial and etcetera. Manuscripts should be submitted to the editorial board of Tungs' Taichung MetroHarbor Hospital Medical Journal:
ADD:No.699, Sec.8, Taiwan Blvd., Wuqi Dist., Taichung City 43503, Taiwan (R.O.C)
E-mail: Tungs_journal@ms.sltung.com.tw

Submission of Manuscripts:

1. The manuscript is required in duplicate (includes an original manuscript and a copy of the original manuscript, however, figures/legends need two original copies) alone with an electronic copy of the latest version; please do not staple the document, if electronic file of figures and/or pictures is being provided, please attached as a JPG format.
2. The content of the document need to be clear, and is consistent with the original copy, the author need to be responsible if the duplicate is different from the original copy or is missing. If the content is related to copyright, author needs to obtain the right to use and is legally responsible for it.
3. Please attached the copyright and consent form on submission. All author(s) listed must actually participate in and agree with the conclusion. Upon receiving and completion of printing, the author(s) will receive 20 free copies and compensation. If extra copy is needed, please notify during editing, and this is subjected to charges.
4. After acquiring consent from the author(s), author(s) agreed to any editing. The submission is returnable, if any, when the manuscript is incompatible with the journal's mission.
5. For any articles listed on the journal, please follow the protocol if related to "the collection of human specimen for research" and "human clinical trail" to ensure the right of participants
6. If the article is related to the use of vertebrates for science application, the project need to be verified by associated animal laboratory management team to ensure the humane management for laboratory animals.

Principles of writing:

1. Original article should be arranged by the following sequence: abstract, introduction, material and methods, results, discussion and conclusion, acknowledgements, reference, attachments, figures and legends for illustration.
2. Case report should be arranged by the following sequence: abstract, introduction, clinical records, discussion, reference, attachments, table, figures and legends for illustration. Each note should be no longer than 5 pages and should be written with the following order: topic, organization, the name of author(s), past history and critical diagnosis, primary clinical problem,

discussion or analysis, conclusion, and references. Patients' eyes should be covered in the picture for privacy. Diagnosis information or the chart of clinical process should be within 6 months.

3. There is no regular form for review articles, but references need to be listed.
4. Images in clinical medicine should be arranged by the following sequence: abstract, figures and legends for illustration.
5. Other types of article should be no longer than 4 pages including charts, except for articles with special arrangement.
6. For other details, please refer to international steering committee, for uniform requirements for manuscripts submitted to biomedical journals please refer to The New England journal of medicine 336:309-315,1997.

Manuscript preparation:

Manuscript should be double-spaced and numbered pages, and comply with the "uniform requirements for manuscripts submitted to biomedical journals". The first page is the title page, which include title, name of author(s), organization and unit, contact name, phone number and address (in both Chinese and English). The second and the third page is for abstract (Chinese content needs to consist with English content) and key words (please include 3 to 5 keywords or phrases in Chinese and English), and should be written in paragraphs following by background and purpose, methods, results and discussion.

Please attach two original copies including attachments, charts and legends. Chart should be professional, with only one figure or one table per page, and is arranged in consecutive orders and numbered in Arabic characters. Table should have a title and appropriate interpretation. Picture should be 5"x 7" in size, black and white, glossy and numbered in consecutive orders of appearance.

Reference:

Unpublished articles or abstracts cannot be listed as references, but could be noted as "unpublished observations". Doctoral dissertation or master thesis can be used. Any articles being accepted by magazines but not published yet, please note the name of magazine, year and note "in press".

Original research, clinical analysis, case reports and special review articles follows the following format:

1. Abbreviations used should follow the format of Index Medicus for all journal titles. When authors are less than 6 people, list all author(s), when more than 6, only list the first 6 followed by "et al" for the rest.
2. References in the text should be placed where relevant. When a reference article is cited, only the primary author is cited; however, if only two authors are present, both should be listed.
3. Example of references:

Examples of Reference:

1. Periodicals:

   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.

2. Monographs:

   Plum F, Posner JB: Diagnosis of Stupor and Coma. 3rd ed. Philadelphia: Davis, 1980:132-3.

3. Monographs with multiple authors:

   Levinsky NG: Fluid and electrolytes. In: Thorn GW, Adams RD, Braunwald E, Isselbacher K, Petersdprf RG eds. Harrison's Principles of Internal Medicine, 8th ed. New York: Mcgraw-Hill, 1977:364-75.

Copyright:

童綜合醫學雜誌投稿相關規則

95.9.01 製訂
99.08.17 修訂
100.07.11 修訂
102.07.08 修訂

本雜誌刊載與醫學有關之論述，包括原著論文、病例分析研究、病例報告等論述及特別約稿之綜論（review article）、special article 、communication（包括 brief communication）、影像判讀、Editorial（編著的話）等。惠稿請送 43503 臺中市梧棲區臺灣大道八段 699 號童綜合醫學雜誌編審委員會。（E-mail:Tungs_Journal@ms.sltung.com.tw）

壹、投稿前注意事項

1. 投稿時，需附原稿兩份（一份原稿和一份複印稿，但圖片應用兩份原圖）並以電腦打字（請以 MS WORD 文書處理格式，中文字型以標楷體，英文字型以 Time New Roman 12 號字大小，稿紙之左右緣爲 2.54 公分，上下緣爲 3.17 公分），請勿裝訂，同時須提供最後版本之電子檔一份，若圖片或照片有電子檔提供者，請以附檔 jpg 的形式提供。
2. 文件內容需清晰，內容與原稿一致，若複印稿與原稿有差異或遺漏，由作者自行負責。著作中若牽扯到版權所有之內容，作者需取得其使用權，法律責任由作者負責。
3. 投稿同時請附上著作權讓與同意書。所有作者必須實際參與並同意該論述。本院於接受稿件且印刷完成後，將致贈稿酬並贈送 20 份抽印本給通訊作者，如需額外抽印本請於校稿時言明，並酌收成本費用 。第一作者若需抽印本可提出申請，依份數酌收成本費用 。
4. 本刊對於原稿經徵得著者之同意得伸縮或修改之。如不合本刊宗旨者，得退還之。
5. 凡刊載於本雜誌之著作，若涉及「研究用人體檢體採集」及「人體試驗」等情事，應遵守該注意事項，以落實保障受檢人權益。詳文請參考須附上相關審議認可之文件。
6. 論文中如涉及使用脊椎動物進行科學應用計畫者，應檢附該計畫業經所屬機構動物實驗管理小組審議認可之文件，以落實實驗動物之人道管理。

貳、寫作原則

1. 原著論文按下列順序撰寫：摘要、前言、材料與方法、結果、討論與結論、誌謝、參考文獻、附表、圖片說明、圖片（含照片）。
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。
4. 綜說不必按原著論文格式撰寫，但必須列出參考文獻。
5. 影像判讀按下列順序撰寫：摘要、圖例說明。
6. 其他類文章連圖表，以不超過四頁（每頁約 2,000 字）爲原則，但特約稿例外。
7. 其他細節，請參閱國際指導委員會（International Steering Committee）發表之生物醫學雜誌稿件統一規格（Uniform Requirements for Manuscripts Submitted to Biomedical Journals，見 The New England Journal of Medicine 336:309-315,1997）。

參、投稿須知

1. 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。
2. 第一頁爲標題頁，須列出中文及英文之論文題目 、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。

3. 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，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）。
4. 請附兩份原稿（一份原稿和一份複印稿，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。

註：[1] 根據「生物醫學雜誌投稿之統一規定」第五版，刊載於 Annals of Internal Medicine 1997;126(1): 36-47.

肆、參考文獻

未經發表之論文或摘要不得列爲參考文獻，但可於本文中說明並註明「未發表」（unpublished observations）。博碩士論文可引用。已被任何雜誌接受刊發但仍未發表之著作，請列出雜誌名稱及年份，並註明「in press」。

原著論文、病例分析研究、病例報告等論述及特別約稿之綜論（review article）按下列格式撰寫：

1. 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 al. The effects of supraphysiologic doses of testosterone on muscle size and strength in normal men. N Engl J Med 1996; 335:1-7.

2. 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

3. 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，同意授權本刊得再授權國家圖書館或其他資料庫業者，進行重製、透過網路提供服務、授權用户下載、列印、瀏覽等行爲。並得爲符合各資料庫之需求，酌作格式之修改。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。

第七卷　第一期　　民國 102 年 01-06 月

童綜合醫學雜誌

綜　論

原　著

病例報告

ISSN 2071-3592

童綜合醫學雜誌

中華民國九十六年十二月創刊
預定出版日期：每年六、十二月三十日出刊

出版編輯部：
童綜合醫學雜誌編審小組
地址：43503 臺中市梧棲區臺灣大道八段 699 號
E-Mail：Tungs_Journal@ms.sltung.com.tw
Tel：〈04〉26581919 ext 58047　Fax：〈04〉26582193

印 刷 者：
大光華印務部　Tel：〈02〉2302-3939（代表號）
地址：10851 台北市萬華區廣州街 32 號 6 樓　Fax：〈02〉2302-2036