Table 3
sFas distribution among smokers according to cigarettes smoked per day.

	Number of cigarettes smoked per day											
	1–5		6–10		11–15		16–20		21–25		26–30		30–	
	N	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%
sFas levels (ng/ml)														
<1.8	12	23.1	60	18.8	82	21.4	193	20.1	27	25.0	57	24.4	22	20.6
1.8–2.2	16	30.8	101	31.6	92	24.0	256	26.6	34	31.5	78	33.3	34	31.8
2.3–2.6	17	32.7	76	23.8	97	25.3	224	23.3	22	20.4	44	18.8	15	14.0
≥2.7	7	13.5	83	25.9	113	29.4	288	30.0	25	23.1	55	23.5	36	33.6
Total	52	100.0	320	100.0	384	100.0	961	100.0	108	100.0	234	100.0	107	100.0
Cochran-Mantel-Haenszel *p* value[a]														$p = 0.26$
Least square means[a] (ng/ml)	2.28		2.37		2.40		2.42		2.34		2.35		2.41	
95% CI	(2.11–2.47)		(2.26–2.48)		(2.29–2.51)		(2.32–2.51)		(2.20–2.49)		(2.24–2.47)		(2.27–2.56)	
p value[b]			0.33		0.19		0.11		0.54		0.44		0.19	

[a] Adjusted for area, age category, BMI, drinking status, walking, education, marital status, consumption of green leaf vegetables.
[b] Compared with smoker who smoked 1–5 cigarettes per day.

sity School of Medicine; Dr. Haruo Mikami, Chiba Cancer Center; Dr. Michiko Kurosawa, Juntendo University School of Medicine; Dr. Yoshiharu Hoshiyama, University of Human Arts and Sciences; Dr. Naohito Tanabe, Niigata University School of Medicine; Dr. Koji Tamakoshi, Nagoya University Graduate School of Health Science; Dr. Kenji Wakai, Nagoya University Graduate School of Medicine; Dr. Shinkan Tokudome, National Institute of Health and Nutrition; Dr. Koji Suzuki, Fujita Health University School of Health Sciences; Dr. Shuji Hashimoto, Fujita Health University School of Medicine; Dr. Shogo Kikuchi, Aichi Medical University School of Medicine; Dr. Yasuhiko Wada, Kansai Rosai Hospital; Dr. Takashi Kawamura, Kyoto University Center for Student Health; Dr. Yoshiyuki Watanabe, Kyoto Prefectural University of Medicine Graduate School of Medical Science; Dr. Kotaro Ozasa, Radiation Effects Research Foundation; Dr. Tsuneharu Miki, Kyoto Prefectural University of Medicine, Graduate School of Medical Science; Dr. Chigusa Date, Faculty of Human Environmental Sciences, Nara Women's University; Dr. Kiyomi Sakata, Iwate Medical University; Dr. Yoichi Kurozawa, Tottori University Faculty of Medicine; Dr. Takesumi Yoshimura, Fukuoka Institute of Health and Environmental Sciences; Dr. Yoshihisa Fujino, University of Occupational and Environmental Health; Dr. Akira Shibata, Kurume University School of Medicine; Dr. Naoyuki Okamoto, Kanagawa Cancer Center; and Dr. Hideo Shio, Moriyama Municipal Hospital.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

The authors express their sincere appreciation to Dr. Kunio Aoki, Professor Emeritus, Nagoya University School of Medicine and a former chairperson of the JACC Study, to Dr. Haruo Sugano, the former Director of the Cancer Institute, Tokyo, who greatly contributed to the initiation of the JACC Study, and to Dr. Yoshiyuki Ohno, Professor Emeritus, Nagoya University School of Medicine, who was also a former chairperson of the study. The authors also wish to thank Dr. Tomoyuki Kitagawa, of the Cancer Institute of the Japanese Foundation for Cancer Research and a former chairperson of a Grant-in-Aid for Scientific Research on Priority Area 'Cancer', for his valuable support of this study.

Funds: The JACC Study has been supported by Grants-in-Aid for Scientific Research (Nos. 61010076, 62010074, 63010074, 1010068, 2151065, 3151064, 4151063, 5151069, 6279102, 11181101, 17015022, 18014011, 20014026) from MEXT, Japan.

References

[1] M. Harel-Meir, Y. Sherer, Y. Shoenfeld, Tobacco smoking and autoimmune rheumatic diseases, Nat. Clin. Pract. Rheumatol. 3 (2007) 707–715.
[2] E. Oudijk, J. Lammers, L. Koenderman, Systemic inflammation in chronic obstructive pulmonary disease, Eur. Respir. J. 46 (2003) s5–s13.
[3] L. Bazzano, J. He, P. Muntner, S. Vupputuri, P. Whelton, Relationship between cigarette smoking and novel risk factors for cardiovascular disease in the United States, Ann. Intern. Med. 138 (2003) 891–897.
[4] D. Yanbaeva, M. Dentener, E. Creutzberg, G. Wesseling, E. Wouters, Systemic effects of smoking, Chest 131 (2007) 1557–1566.
[5] J. Feldkamp, E. Pascher, M. Schott, P. Goretzki, J. Seissler, W. Scherbaum, Soluble Fas is increased in hyperthyroidism independent of the underlying thyroid disease, J. Clin. Endocrinol. Metab. 86 (2001) 4250–4253.
[6] J. Hao, D. Ye, G. Zhang, H. Liu, H. Dai, F. Huang, F. Pan, H. Su, M. Dong, H. Chen, Q. Wang, X. Zhang, Elevated levels of serum soluble Fas are associated with organ and tissue damage in systemic lupus erythematosus among Chinese, Arch. Dermatol. Res. 297 (2006) 329–332.
[7] M.J. Hebert, M. Masse, N. Vigneault, I. Sirois, S. Troyanov, F. Madore, Soluble Fas is a marker of coronary artery disease in patients with end-stage renal disease, Am. J. Kidney Dis. 38 (2001) 1271–1276.
[8] S. Troyanov, M.J. Hebert, M. Masse, N. Vigneault, I. Sirois, F. Madore, Soluble Fas: a novel predictor of atherosclerosis in dialysis patients, Am. J. Kidney Dis. 41 (2003) 1043–1051.

[9] C. Nathan, Points of control in inflammation, Nature 420 (2002) 846–852.
[10] M. Iwase, G. Kondo, H. Watanabe, S. Takaoka, M. Uchida, M. Ohashi, M. Nagumo, Regulation of Fas-mediated apoptosis in neutrophils after surgery-induced acute inflammation, J Surg. Res. 134 (2006) 114–123.
[11] L. Doughty, R. Clark, S. Kaplan, H. Sasser, J. Carcillo, sFas and sFas ligand and pediatric sepsis-induced multiple organ failure syndrome, Pediatr. Res. 52 (2002) 922–927.
[12] E. Papathanassoglou, J. Moynihan, D. Vermillion, M. McDermott, M. Ackerman, Soluble Fas levels correlate with multiple organ dysfunction severity, survival and nitrate levels, but not with cellular apoptotic markers in critically ill patients, Shock 14 (2000) 107–112.
[13] Y. Ohno, A. Tamakoshi, JACC Study Group, Japan Collaboraetive Cohort Study for evaluation of cancer risk sponsored by Monbusho (JACC Study), J. Epidemiol. 11 (2001) 144–150.
[14] A. Tamakoshi, T. Yoshimura, Y. Inaba, Y. Ito, Y. Watanabe, K. Fukuda, H. Iso, JACC Study Group, Profile of the JACC Study, J. Epidemiol. 15 (2005) S4–S8.
[15] A. Tamakoshi, K. Nakachi, Y. Ito, Y. Lin, K. Yagyu, S. Kikuchi, Y. Watanabe, Y. Inaba, K. Tajima, JACC Study Group, Soluble Fas level and cancer mortality: findings from a nested case-control study within a large-scale prospective study, Int. J. Cancer 123 (2008) 1913–1916.
[16] Y. Ito, K. Nakachi, K. Imai, S. Hashimoto, Y. Watanabe, Y. Inaba, A. Tamakoshi, T. Yoshimura, Stability of frozen serum levels of insulin-like growth factor-I, insulin-like growth factor-II, insulin-like growth factor binding protein-3, transforming growth factor β, soluble Fas, and superoxide dismutase activity for the JACC study, J. Epidemiol. 15 (2005) S67–S73.
[17] S. Holdenrieder, P. Stieber, Apoptotic markers in cancer, Clin. Biochem. 37 (2004) 605–617.
[18] K. Nozawa, N. Kayagaki, Y. Tokano, H. Yagita, K. Okumura, H. Hasimoto, Soluble Fas (APO-1, CD95) and soluble Fas ligand in rheumatic diseases, Arthritis Rheum. 40 (1997) 1126–1129.
[19] P. Imirzalioglu, S. Uckan, E.E. Alaaddinoglu, A. Haberal, D. Uckan, Cigarette smoking and apoptosis, J. Periodontol. 76 (2005) 737–739.
[20] A.J. Cox, D.B. Pyne, M. Gleson, R. Callister, Resting plasma and salivary IL-6 concentrations are not correlated in distance runners, Eur. J. Appl. Physiol. 103 (2008) 477–479.
[21] I. Hayashi, Y. Morishitab, K. Imai, M. Nakamura, K. Nakachi, T. Hayashi, High-throughput spectrophotometric assay of reactive oxygen species in serum, Mutat. Res. 631 (2007) 55–61.
[22] M. Ohsawa, A. Okayama, M. Nakamura, T. Onoda, K. Kato, K. Itai, Y. Yoshida, A. Ogawa, K. Kawamura, K. Hiramori, CRP levels are elevated in smokers but unrelated to the number of cigarettes and are decreased by long-term smoking cessation in male smokers, Prev. Med. 41 (2005) 651–656.
[23] S. Wannamethee, G. Lowe, A. Shaper, A. Rumley, L. Lennon, P. Whincup, Associations between cigarette smoking, pipe/cigar smoking, and smoking cessation, and haemostatic and inflammatory markers for cardiovascular disease, Eur. Heart J. 26 (2005) 1765–1773.

総　　論

H. pylori 感染症の疫学と感染経路の解明

菊地正悟　林　櫻松

Epidemiology of *H. pylori* infection and exploration of its infection route

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Abstract

To build up a preventive strategy for *H. pylori* related diseases including gastric cancer, infection ages and routes are important. Recently, several studies have explored them. In advanced countries, most infections occur under five years of age. Mother to child in some studies and sibling to sibling in others were dominant infection routes. As infection ages and routes depend on countries, studies in Japan are indispensable. Infection in kindergartens, nursery and elementary schools as well as in families should be explored. Based on the findings, a strategy preventing infection to children should be build up to prevention *H. pylori* related diseases.

Key words: intra-familial infection, infection in kindergarten or school, DNA fingerprinting, infection route, infection age

はじめに

H. pylori 感染は胃癌を含めた多くの疾患の原因となることが明らかにされている．*H. pylori* 感染の防止によるこれらの疾患の予防を考えるうえで，*H. pylori* 感染症の疫学，特に感染経路は重要である．胃癌については，一度も感染したことのない者に比べ，感染者では20倍以上胃癌のリスクが高いことが明らかになっており[1]，感染防止ができれば胃癌は稀少がんとなることが予想されている．*H. pylori* 感染症の疫学，感染経路について，最近の報告を中心にreviewするとともに，今後の研究の方向性について検討した．

1．*H. pylori* 感染症の疫学

H. pylori の感染率にはっきりした性差はない．年齢別には，**図1**に示した某町の健診受診者の血清抗体陽性率[2]のように，高齢ほど陽性率が高くなる．*H. pylori* は一度持続感染が成立すると，除菌や胃粘膜の強度萎縮などの環境変化がないかぎり感染が持続するので，感染の累積によって年齢とともに感染率が上昇する．また，後述するように小児期の衛生環境が感染率に大きく影響するので，経済発展による社会基盤の整備がなされると後から生まれる世代ほど感染率は低下する（コホート効果）．感染の累積に加え，コホート効果によっても年齢とともに感染率が上昇する．**図1**では2003年の方が1997年

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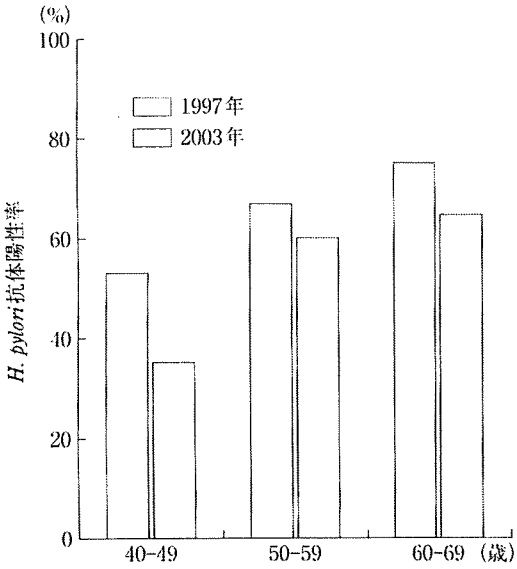

図1　某町の基本健康診査受診者の *H. pylori* 抗体保有率

表1　これまでに明らかにされた *H. pylori* の感染時期，感染経路

主な感染時期は小児期である．
小児期の衛生環境*が悪いと感染率が高い．
保育園，幼稚園の通園期間が長いと感染率が高い．
低い社会階層に属するほど感染率が高い．

*上下水道の整備状況，1人あたりの部屋数などの居住環境など．

より同じ年齢での感染率が低くなっており，この低下は我が国の社会基盤の整備によるコホート効果によると考えられる．

コホート効果による感染率の減少は，欧米の先進国では我が国に先立って起こったと推定される．一方，現在経済発展が著しい諸国でも，コホート効果による若い世代での感染率の低下が観察されている．

2. 感染経路に関する既存の知見

H. pylori の感染経路に関しては，この細菌の発見以来これまで多くの研究がなされてきた．その中で，明らかにされてきたことは，**表1**に示すような内容である．

H. pylori の特異的な感染経路については，不完全な消毒の内視鏡による感染以外，いまだ不明である．ヒトが日常接する環境中からはほとんど検出されないので，ヒト→ヒト感染が主な経路と考えられている．胃の粘液が生息場所であるので，経口感染することは間違いないとされているが，口→口感染であるか便→口感染であるかは明らかでない．

3. 感染年齢

主な感染時期は小児期であるが，その中でどの年齢での感染が多いかが注目されている．これは，除菌後の再感染の可能性がどのくらいあるかなど，適切な除菌時期を考えるうえで重要だからである．

Rowland らは，ダブリン(アイルランド)での研究で年齢別の新規感染率を観察した結果を報告している[3]．279 人の小児を追跡した結果，2-3 歳で 5 人/99 人年(以下同じ)，3-4 歳で 9/214，4-5 歳で 5/241，5-6 歳で 0/224，6-7 歳で 1/146，7-8 歳で 0/46 と，主な感染時期は 5 歳以下であるが，6-7 歳でも感染が観察されている．

Konno らは，*H. pylori* 抗体陽性の母親から生まれた 44 人の便中 *H. pylori* 抗原を用いて 5 年間追跡調査し，5 人の感染児を確認した．感染時期は 1 歳が 4 人，4 歳が 1 人であった[4]．

Okuda らは，乳児 237 人の感染時期を便中抗原検査によって追跡調査した結果，5 人の感染を確認し，感染時期は乳児 3 人，1 歳，2 歳がそれぞれ 1 人であった[5]．

これらの追跡調査から先進国における主な感染時期は 5 歳頃までと推測される．しかし，ダブリンで 6 歳での感染の報告や，我が国の他の研究でも，6 歳や 10 歳代での感染が報告されるなど，例外も少なくない．

4. 感染予防に向けた感染経路の研究

H. pylori の特異的な感染経路の特定が難しいことから，広めの範囲に網をかけて感染を防止することが考えられるようになっている．*H. pylori* については，感染しているヒトが感染源であるが，除菌をすることで感染源でなくすることが可能である．最近の内外の研究には，この視点に立っていると思われるものが散見され

表2 小児における*H. pylori*感染経路の分類

1. 家族内感染
   - 母 → 子
   - 父 → 子
   - 同胞 → 同胞
2. 集団生活での感染(保育園・幼稚園・学校)
   - 児童/生徒 → 児童/生徒
   - 教員/職員 → 児童/生徒
3. その他
   - 家族や集団生活以外の人から
   - 環境中から

る．これらの研究は，**表2**に示すような分類の経路での感染が，それぞれどのくらいの比重であるのかを明らかにすることを目的としている．

Farrellらは，ベルファスト(英国)で52家族(小児は126人)の*H. pylori*感染を調査し，母の感染，父の感染，同胞(兄弟姉妹)との部屋の共有，同胞とのベッドの共有のどれが最も強く小児の感染に関連するかを分析した．オッズ比(95％信頼区間，以下95％ CI)は母の感染では2.5(1.0-6.1)，父の感染では3.9(1.0-8.6)，同胞との部屋の共有では3.7(1.3-10.8)，同胞とのベッドの共有では4.8(1.5-15.2)であった．同胞とのベッドの共有が最も関連が強く，この経路による感染の頻度が高いとしている[6]．家族内感染のうち，同胞間感染の比重が大きいことを示す結果である．

Weyermannらは，ウルム(ドイツ)で612人の3歳児とその両親の*H. pylori*感染を調べ，児の感染と両親の感染の関連を分析した．国籍やもう片方の親の感染の有無で補正すると，母親の感染はオッズ比13(95％CI: 3.2-52.5)で有意な関連を示したが，父親の感染は1.4(95％CI: 0.4-4.6)と有意ではなかった．このことから，父子感染に比べ，母子感染優位であると結論している[7]．

またWeyermannらは，メタアナリシスの手法で493例の4歳児の*H. pylori*感染の有無と，その家族の感染の関係を分析した．両親の民族，配偶者の感染と同胞の感染の有無，同胞の有無で補正したオッズ比(95％CI)は，母の感染の有無13.0(3.0-55.2)，父の感染の有無3.0(0.8-11.2)，同胞の1人以上が感染あり3.7(0.5-26.2)と，母の感染の有無だけが有意であった[8]．ウルムでの研究と同様に，母から子への感染の頻度が高いことを示す結果である．

Kiviらは，ストックホルム(スウェーデン)で，同一家族内で*H. pylori*感染陽性の者同士で*H. pylori*の株(strain)が一致するかを分析した．一致率(一致した組数/分析した組数)は，同胞同士81％(29/36)，母がいずれとも異なる株に感染している同胞同士82％(14/17)，母子56％(10/18)，父子0％(0/8)，夫婦22％(5/23)であった[9]．親から子への感染よりも同胞間での感染が主であることを示す結果である．

Konnoらは，前述した研究での5人の感染児とその母親に感染している*H. pylori*が同一起源であるかを確認するために，Random amplified polymorphic DNA fingerprinting(RAPD)法によるDNAパターンの分析を行った．その結果，5例全例ともその母親とRAPD法によるDNAパターンが一致しており，すべて母子感染であったと結論している[4]．

Konnoらは更に，上部消化管内視鏡検査と生検組織で診断された小児の*H. pylori*胃炎42例を発端児として，家族内で*H. pylori*のDNAパターンが一致するかRAPD法によって分析した．家族に便中抗原検査HpSAを行い，陽性者からは胃液の採取，希望者には内視鏡検査を施行して胃粘膜を採取し，菌株を培養してRAPD法による分析を行った．家族の*H. pylori*陽性率(陽性数/検査数)は，母が86％(36/42)，父が82％(32/39)，同胞が43％(18/42)であった．DNAパターンの一致率(一致した組数/分析した組数)は，母子で69％(29/42)，父子で37％(7/19)で，父子間に比べ母子間で有意に高かった．発端児と同胞の一致率は全体で80％(8/10)，母親と発端児のDNAパターンが一致する家族では88％(7/8)であった(1家族で発端児と他の2人の同胞が一致する例を含む)．また，発端児42例中32例(76％)で，家族のいずれか(祖母と一致した1例を含む)とDNAパターンが一致した[10]という詳細な結果を報告している．

これらの結果は，**表2**の家族内感染の部分に

関するものである．家族内感染が小児における感染の主要な部分を占めていること，父子感染に比べれば母子感染の頻度が高いことも間違いない．しかし，母子感染と同胞間感染に関しては結果が分かれている．

5. *H. pylori* の除菌による胃癌の予防

胃癌予防の方法の一つとして，一定の年齢に達した段階で感染の有無を確認して感染者の除菌を行うという方法が考えられる．除菌の時期は早い方が感染期間が短いので，より効果的である．一方，除菌治療の安全性は小児では確立されていないため，無症状の場合の除菌はできるだけ成人に近い年齢で，あるいは成人になってから行うべきである．更に，再感染の頻度を考慮して除菌の年齢を決める必要がある．

この方法には，感染者は一定期間感染を受けることになるという欠点があるが，対象の選択が容易で実施しやすい方法と考えられる．

6. *H. pylori* の感染防止

もう一つの方法として，小児期の感染自体を防止するものがある．まず，**表2**に示す各感染経路の比重を明らかにする．ほとんどの感染が小児期に起こることから，仮に家族内感染以外に子への感染がないとすると，第1子出産以前の段階で同居家族の感染者を除菌すれば子への感染は防止できることになる．また，家族内感染と集団生活での感染以外に子への感染がなかったとすると，第1子出産以前の段階での同居家族の感染者の除菌と保育園，幼稚園，学校の職員の感染者を除菌することで，子への感染は防止できることになる．

生活習慣や育児方法は，小児での *H. pylori* 感染に影響を与えていると思われるが，国や地域などによって異なる．同胞数も感染経路に影響を与えている可能性が高く，先進国と発展途上国では同胞数が異なる．母子感染が優位であるという報告と同胞間感染が優位であるという報告があることも，これらの要因の国や地域による違いで説明できる．我が国において，小児期の感染防止を考えるうえでは，我が国で感染がどのように起こっているのかを明らかにすることが不可欠である．我が国で家族内感染を詳細に検討したデータはKonnoらの研究[10]だけである．地域を変えて，同様の研究を行うことにより，我が国における家族内感染の実態を十分なデータで明らかにしていく必要がある．

上下水道の整備状況と *H. pylori* の陽性率に負の関連がある[11]ことから，先進国では環境からの感染は少なく，大部分が家族内と集団生活での感染であると推測される．我が国においても家族内と集団生活以外での感染は，少なくなっていることが予測される．家族内感染より対象を広げて，保育園児，幼稚園児，小学生の *H. pylori* 感染者について，その家族と接触のあった教職員を含めてDNAパターンを検査し，どのような一致がみられるかを明らかにすることも，今後の研究の方向性として重要である．家族内と集団生活以外での感染が極めてまれであることが明らかとなれば，前述したような対策で次の世代への感染を0に近づけることが可能となる．

このような感染防止の方法は，感染検査の対象の選択が複雑で，情報の管理に細心の注意を要するなど実施がやや難しい方法である．しかし，感染そのものを防止するので，除菌による方法に比べ胃癌などの疾患の予防効果は確実であり，検討すべき方法であると思われる．

おわりに

H. pylori の感染防止や除菌による，胃癌などの *H. pylori* 関連疾患の予防を考えるうえで，年齢ごとの感染頻度や感染経路を明らかにすることが重要である．これまでの報告で，我が国などの先進国では5歳までが主な感染時期であるが例外もあることが明らかとなっている．感染経路に関しては，母子感染が優位という報告と同胞間感染が優位という報告がある．感染年齢や感染経路は国によって異なる．我が国ではこの分野の研究が少ない．今後，対策を構築するうえでは，根拠となりうる十分なデータを我が国で集める必要がある．

文　献

1) Ekstrom AM, et al: *Helicobacter pylori* in gastric cancer established by CagA immunoblot as a marker of past infection. Gastroenterology **121**: 784-791, 2001.
2) 菊地正悟：わが国における *Helicobacter pylori* 感染の推移と若年胃癌の実態. Helico Res **9**: 411-416, 2005.
3) Rowland M, et al: Age-specific incidence of *Helicobacter pylori*. Gastroenterology **130**: 65-72, 2006.
4) Konno M, et al: Five-year follow-up study of mother-to-child transmission of *Helicobacter pylori* infection detected by a random amplified polymorphic DNA fingerprinting method. J Clin Microbiol **43**: 2246-2250, 2005.
5) Okuda M, et al: *Helicobacter pylori* colonization in the first 3 years of life in Japanese children. Helicobacter **12**: 324-327, 2007.
6) Farrell S, et al: Risk factors for *Helicobacter pylori* infection in children: an examination of the role played by intrafamilial bed sharing. Pediatr Infect Dis J **24**: 149-152, 2005.
7) Weyermann M, et al: The mother as a source of *Helicobacter pylori* infection. Epidemiology **17**: 332-334, 2006.
8) Weyermann M, et al: Acquisition of *Helicobacter pylori* infection in early childhood: independent contributions of infected mothers, fathers, and siblings. Am J Gastroenterol **104**: 182-189, 2009.
9) Kivi M, et al: Concordance of *Helicobacter pylori* strains within families. J Clin Microbiol **41**: 5604-5608, 2003.
10) Konno M, et al: Predominance of mother-to-child transmission of *Helicobacter pylori* infection detected by random amplified polymorphic DNA fingerprinting analysis in Japanese families. Pediatr Infect Dis J **27**: 999-1003, 2008.
11) 植田美津江ほか：*Helicobacter pylori* の感染要因. *Helicobacter* Research **5**: 599-602, 2001.

Journal of Viral Hepatitis, 2009 doi:10.1111/j.1365-2893.2009.01243.x

Risk factors for intrahepatic cholangiocarcinoma: a possible role of hepatitis B virus

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Received July 2009; accepted for publication October 2009

SUMMARY. There are several established risk factors for intrahepatic cholangiocarcinoma (ICC), namely primary sclerosing cholangitis, fibropolycystic liver disease, parasitic infection, intrahepatic biliary stones and chemical carcinogen exposure. However, the majority of patients with ICC do not have any of these risk factors. Therefore, identification of other risk factors is warranted for the prevention and early detection of ICC. We evaluated the risk factors for ICC in a large-scale cohort study in the province of Osaka, Japan. This retrospective cohort study included 154,814 apparently healthy individual blood donors, aged 40–64 years at the time of blood donation in the period 1991–1993. The average observation period was 7.6 years, resulting in 1.25 million person-years of observation. Incident ICC cases were identified by linking the blood-donor database to the records in the population-based cancer registry for the province. There were 11 incident ICC cases during follow-up, with an incidence rate of 0.88 per 100 000 person-years. Compared with subjects aged 40–49 years, the subjects aged 50–54 years and 55–59 years had a significantly higher risk for ICC (hazard ratio [HR] = 5.90; 95%CI:1.08–32.31 and 11.07; 95%CI:1.98–61.79, respectively). Compared with those with ALT level of 19 Karmen Units (KU) or less, subjects with ALT level of 40 KU or higher had a significantly higher risk for ICC (HR: 8.30; 95%CI:1.47–46.83). Compared with those who tested negative for both HBsAg and anti-HCV, those who tested HBsAg-positive had a significantly higher risk for ICC (HR: 8.56; 95%CI: 1.33–55.20). Our results suggest that HBV infection and liver inflammation are independently associated with ICC development. These findings need to be verified by further large cohort studies.

Keywords: aetiology, cholangiocarcinoma, cohort studies, hepatitis, inflammation, liver.

INTRODUCTION

Intrahepatic cholangiocarcinoma (ICC) is the second most common primary liver cancer, accounting for approximately 10–20% of liver cancers [1]. Worldwide, it is estimated to account for 3% of all gastrointestinal cancers [2]. Advanced ICC has a very poor prognosis with a median survival of less than 24 months [3]. There is extensive variation among the incidence rates of ICC in different parts of the world, and the incidence is reported to be higher in East Asia [1]. The reported incidence of ICC in several developed countries has been increasing in recent years [1,4,5].

Abbreviations: HBsAg, Hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; anti-HCV, antibody to HCV; HR, hazard ratio; HTLV-1, human T-cell lymphotropic virus type 1; ICC, intrahepatic cholangiocarcinoma; KU, Karmen Unit; OCR, Osaka Cancer Registry.

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There are several established risk factors for ICC [1,4], namely primary sclerosing cholangitis, fibropolycystic liver disease, parasitic infection, intrahepatic biliary stones and chemical carcinogen exposure, but the majority of patients with ICC do not have any of these risk factors. Therefore, identification of other risk factors is warranted for the prevention and early detection of ICC. In addition to the established risk factors mentioned earlier, some other potential risk factors for ICC have been suggested, such as infection with hepatitis B virus (HBV) [6–9], hepatitis C virus (HCV) [9–14] or liver cirrhosis [7,11,12]. Almost all of the studies that suggested these potential risk factors, however, were case–control studies. Also, half of the reported studies were conducted in the USA or Italy, and their results may not be applicable for populations in other countries or areas, partly because the relative importance of risk factors may vary by country or by area. To our knowledge, no cohort study has been reported from a non-Western country on the risk factors for ICC. In this background, we conducted a retrospective cohort study in Japan, using a large group of

apparently healthy blood donors, to assess the incidence and risk factors for ICC.

MATERIALS AND METHODS

Subjects

The subjects were those who were involved in our previous study on the risk of developing hepatocellular carcinoma, which was reported elsewhere [15]. They were selected from voluntary blood donors who gave 1 235 926 allogeneic blood donations at the Osaka Red Cross Blood Center between 1991 and 1993. The blood centre is in charge of managing volunteer blood donations in the province of Osaka, which had a population of approximately 8.6 million during this period. Conditions required for potential blood donors in Japan during the study period were described elsewhere [16]. In brief, they had to be aged between 16 and 64 years and have a haemoglobin level of 12 g/dL or higher. Potential blood donors were preliminarily screened by a self-administered questionnaire with items regarding past and present illnesses, history of blood transfusion, illegal drug use and high-risk sexual behaviours. Major exclusion criteria in the preliminary screening included: (i) known or potential infection with HBV, HCV, human immunodeficiency virus (HIV) or human T-cell lymphotropic virus type 1 (HTLV-1); (ii) presence or history of chronic liver disorders as well as malignant, allergic or autoimmune disorders; (iii) history of illegal drug use or high-risk sexual behaviour. Those who did not meet any of the exclusion criteria in the preliminary screening donated blood without monetary incentive. The donated blood of these donors was tested for the earlier-described viruses, and, if tested positive, the blood was discarded, but the information on the positive-tested donors was kept in the blood-donor database as was the information on the negative-tested donors. Using this database containing information on all of the donors and the results of the serologic screening test, we identified 667 461 individual donors by donor birthdate, sex, first name, family name and ABO blood type. From these individuals, we selected residents in Osaka province, aged 40 years or older at the time of blood donation, who were negative for antibodies to HIV and HTLV-1. Those who were 39 years or younger were excluded because the incidence of ICC in this age-group in the Japanese population was negligible. Those infected with HCV or HBV were first-time blood donors who claimed to be asymptomatic at the time of blood donation. Those who were infected with both HBV and HCV (25 subjects) were also excluded. As a result, we identified 154 814 persons to be included in this cohort study.

Blood screening tests

The serum alanine aminotransferase (ALT) level expressed in Karmen Units (KU) and serum total cholesterol level (mg/dL) were obtained from the database. The ALT value in KU tested by the blood centre can be translated into a value in International Units by multiplying with 1.5 [17]. An individual was defined as having HCV infection if the titre of anti-HCV in a second-generation passive hemagglutination assay (PHA) (Dainabot Co., Ltd, Tokyo, Japan) was 2^{12} or higher, because the positive predictive value for being HCV-RNA-positive using this cut-off point in Japanese blood donors was guaranteed [18]. An individual was considered to have HBV infection if he/she was positive for HBsAg in a reverse passive hemagglutination assay (Japan Red Cross, Tokyo, Japan).

Follow-up

The subjects were followed-up by record linkage between the blood-donor database and the database of the Osaka Cancer Registry (OCR) [19]. The records in the two databases were linked by the parameters of sex, date of birth, address and the first character of the family name in Chinese letters. The OCR is a population-based cancer registry that covers all of the population in Osaka province. The OCR registers all incident cancer cases using reports from health care facilities in the province as well as death certificate information provided by the Osaka Provincial Government [19]. In the OCR database, ICC cases were identified by the ICD-10 code (C22.1). The diagnosis of ICC was based on histological examination and/or combined clinical, radiological (echography, computed tomography and endoscopic retrograde cholangio-pancreatography) and laboratory findings. Subjects who remained unaffected by ICC were censored at the last date of follow-up, 31 December 2000, as were the subjects in the previous study [15]. The study protocol was approved by both the Ethical Committee of Osaka Medical Center for Cancer and Cardiovascular Diseases, and the Ethical Committee of the Osaka Red Cross Blood Center.

Statistical analyses

The number of person-years of observation of the subjects was determined, and the incidence rate of ICC per 100 000 person-years was calculated by the strata of age-group, sex, ALT level, cholesterol level and HBV/HCV infection status. Ninety-five per cent confidence interval (95%CI) for the rate was calculated using Byar's approximation of the exact Poisson test. Independent factors associated with the development of ICC were analysed by Cox proportional hazards model, and hazard ratios were calculated with 95%CI. In the model, age-group, sex, ALT level, cholesterol level, and HBV/HCV infection were included as independent variables. Data analyses were performed with the SAS/PC statistical package (SAS Institute, Cary, NC, USA).

RESULTS

The baseline characteristics of the 154 814 study subjects and incidence rates of ICC stratified by different characteristics

are shown in Table 1. The proportions of those with ALT level of 40 KU or higher, with positive HBsAg test, and with positive anti-HCV test were 2.2%, 1.6% and 1.2%, respectively. The average observation period was 7.6 years, resulting in 12.50×10^5 person-years. There were 11 incident ICC cases, with an incidence rate of 0.88 per 100 000 person-years (95%CI: 0.44–1.58). By strata, the point-estimate incidence rate was higher among those aged 55–59 years, those with ALT level of 40 KU or higher, or those who tested positive for HBsAg or anti-HCV.

Factors associated with the development of ICC in blood donors are shown in Table 2. Compared with subjects aged 40–49 years, the subjects aged 50–54 years and 55–59 years had a significantly higher risk for ICC. Compared with those with ALT level of 19 KU or lower, subjects with ALT level of 40 KU or higher had a significantly higher risk for ICC. Compared with those who tested negative for both HBsAg and anti-HCV, those who tested positive for HBsAg had a significantly higher risk for ICC. The hazard ratio for anti-HCV positivity was 2.63, although it was not significant.

The characteristics of the 11 ICC cases identified during the follow-up period are summarized in Fig. 1. The observation period from the date of blood donation to the date of diagnosis of ICC ranged from 14 to 90 months among the eight cases not infected with HBV or HCV, while it was '41', '77' and '108' months in the one HCV- and two HBV-infected cases.

DISCUSSION

To our knowledge, this is the first cohort study that investigated the risk factors for ICC in East Asia, where the incidence of primary liver cancer is high [20]. A literature search in Medline from January 1992 up to May 2009 revealed that all of the analytical studies on the risk factors for ICC in the past employed the case–control design, except for one study (Table 3). The only cohort study in the past was reported from the USA in early 2009 and focused on the risk of HCV infection for ICC and other hepatobiliary carcinomas [10]. The present study assessed whether HBV infection or liver inflammation as expressed by the ALT level was independently associated with ICC, which was not scrutinized in the cohort study from the USA.

We found that the incidence rate of ICC among the apparently healthy population aged 40–64 years was

Table 1 Baseline characteristics of the study subjects of blood donors aged 40–64 years and incidence rates of intrahepatic cholangiocarcinoma.

	N	(%)	ICC incident cases	Person-years ($\times 10^5$)	Incidence rate of ICC (per 10^5 person-years)	95% Confidence interval* of ICC incidence rate (per 10^5 person-years)
All cases	154 814	100.0	11	12.50	0.88	0.44–1.58
Age at blood donation (years)						
40–49	90 223	58.3	2	7.29	0.27	0.03–0.99
50–54	35 308	22.8	4	2.85	1.41	0.38–3.59
55–59	20 668	13.4	4	1.67	2.40	0.64–6.13
60–64	8615	5.6	1	0.69	1.44	0.02–8.06
Sex						
Male	84 205	54.4	7	6.81	1.03	0.41–2.12
Female	70 609	45.6	4	5.68	0.70	0.19–1.80
ALT (KU)						
19 or lower	127 757	82.5	7	10.32	0.68	0.27–1.40
20–39	23 666	15.3	2	1.91	1.05	0.12–3.78
40 or over	3391	2.2	2	0.27	7.36	0.83–26.74
Cholesterol (mg/dL)						
139 or lower	4533	2.9	0	0.37	0.00	0.00–12.60
140–199	82 575	53.3	8	6.67	1.20	0.52–2.36
200 or over	67 706	43.7	3	5.46	0.55	0.11–1.61
Hepatitis B/C virus infection						
HBsAg+	2519	1.6	2	0.22	9.08	1.02–32.82
anti-HCV+	1927	1.2	1	0.16	6.34	0.08–34.77
All negative	150 368	97.1	8	12.12	0.66	0.28–1.30

None of the subjects was positive for human immunodeficiency virus or human T-cell lymphotropic virus type 1. All negative: tested negative for both anti-HCV and HBsAg. ALT, alanine aminotransferase; HBsAg+, tested positive for Hepatitis B surface antigen and negative for Hepatitis C virus antibody; anti-HCV+, tested positive for anti Hepatitis C virus antibody and negative for Hepatitis B surface antigen. *95% confidence interval was calculated by Byar's approximation of the exact Poisson test.

Table 2 Factors associated with the development of intrahepatic cholangiocarcinoma in blood donors according to Cox proportional hazard analysis

Variable	*n*	ICC	Hazard ratio	95%CI
Age at blood donation (years)				
40–49	90 223	2	1.00	
50–54	35 308	4	5.90	1.08–32.31
55–59	20 668	4	11.07	1.98–61.79
60–64	8615	1	6.61	0.59–74.59
Sex				
Male	84 205	7	1.00	
Female	70 609	4	0.79	0.22–2.82
ALT level (KU) at blood donation				
19 or lower	127 757	7	1.00	
20–39	23 666	2	1.47	0.29–7.36
40 or over	3391	2	8.30	1.47–46.83
Cholesterol level at blood donation*				
200 mg/dL or higher	67 706	3	1.00	
140–199	82 575	8	2.36	0.60–9.26
139 or lower	4533	0	–	–
Hepatitis B/C virus infection				
All negative	150 368	8	1.00	
HCV-Ab +	1927	1	2.63	0.25–27.73
HBs Ag+	2519	2	8.56	1.33–55.20

None of the subjects was positive for human immunodeficiency virus or human T-cell lymphotropic virus type 1. Age (4 categories), sex, serum ALT level at blood donation (3 categories), and serum cholesterol level at blood donation (3 categories) were included as independent variables in the Cox proportinal hazard analysis. ALT, alanine aminotransferase; Cl, confidence interval; HBsAg+, tested positive for Hepatitis B surface antigen and negative for Hepatitis C virus antibody; HCV-Ab+, tested positive for anti Hepatiis C virus antibody and negative for Hepatitis B surface antigen; ICC, intrahepatic cholangiocarcinoma; KU, Karmen Unit.

roughly 1 ± 0.5 per 100 000 person-years in Osaka where the HCV carrier rate is relatively high [21]. This figure is higher than the estimated incidence rate of 0.037 in the population aged 40–69 years reported in another Japanese study [22], of which the rate was underestimated because the numerator was derived from a multi-centre survey of primary liver cancer across Japan [23,24] with limited population coverage. The point-estimate incidence rate of ICC among anti-HCV positive subjects in our study (6.34 per 100 000 person years) was not very different from the estimate in the cohort study from the USA (4.0 per 100 000 person years) [10], even though our confidence interval was quite large.

Our results suggest that HBV infection is likely to be an independent risk factor for ICC in Japan. Studies in the past, in aggregate, suggested that HBV and HCV infection are potential risk factors for ICC, but their impact might be different across different countries or areas (Table 3). Several studies from the USA and Italy [9–12,14] consistently found that HCV infection was significantly associated with ICC, while the association with HBV infection was inconsistent or not assessed. On the other hand, recent hospital-based case–control studies from Korea and Shanghai, China [7,8] found that not HCV but HBV infection was significantly associated with ICC. The most recent study from Taiwan found that both HBV and HCV are significantly associated with ICC [6]. To our knowledge, our study is the first cohort study to demonstrate a significant association between HBV infection and ICC. This higher level of evidence supports prior observations of the association in East Asia. Our finding is also supported by the results of the earlier mentioned multi-centre primary liver cancer survey in Japan, which consistently showed a high prevalence of HBsAg among ICC cases (4–9%) since 1990 [24][detailed data since 1990 available in reports by the The Japan Society of Hepatology (Japanese only)].

The mechanism of carcinogenesis by HBV in intrahepatic bile ducts has not yet been elucidated, but HBV infection is an established risk factor for hepatocellular carcinoma. Because both hepatocytes and cholangiocytes differentiate from the same progenitor cells, HBV might induce carcinogenesis in both cell types through the same mechanism. The HBV gene has been detected in cholangiocarcinoma tissue in some studies [25,26], and its presence has been associated with the potential of carcinogenesis in human cholangiocytes [27]. Alternatively, hepatitis-associated ICC may arise from hepatic progenitor cells, as suggested by Lee *et al.* [6].

In our study, the association between ICC and HCV infection was not significant, even though its hazard ratio

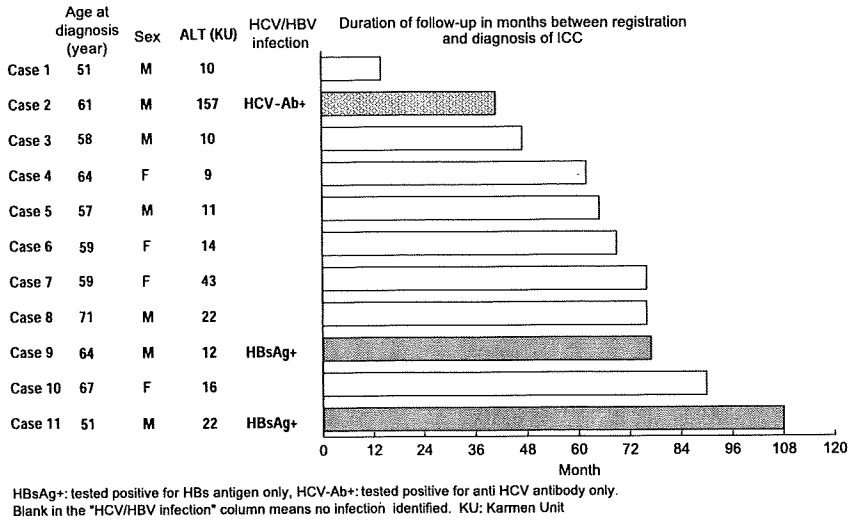

Fig. 1 Characteristics of the 11 intrahepatic cholangiocarcinoma (ICC) cases identified among the cohort of blood donors during the follow-up period.

was greater than unity. We might have seen a significant association if our cohort had been even larger. In the multicentre survey in Japan, the prevalence of anti-HCV among ICC cases (20–30%) has consistently been high since 1990 [24]. [detailed data since 1990 available in reports by The Japan Society of Hepatology (Japanese only)].

Our findings also suggest that liver inflammation as expressed by ALT level ≥40 KU may be an independent risk factor for ICC after adjustment for HCV and HBV infection status. In our previous study using the same cohort, we also found that liver inflammation is an independent risk factor for hepatocellular carcinoma [15]. The only study in the past that assessed the impact of ALT level on the development of ICC was performed in a case–control study [13], and found a significant association between ICC and ALT level of ≥40 IU, independent of HBV or HCV infection status. In addition to viral hepatitis, fatty liver disease is likely to be a major cause of liver inflammation in the subject population. Here, inflammation of hepatocytes may connote inflammation of intrahepatic bile ducts. Epidemiologic and experimental evidence shows that inflammation of the bile duct from various aetiologies induces carcinogenesis in cholangiocytes [1,4,28]. Alternatively, inflammation of hepatic progenitor cells may result in cholangiocarcinoma [6].

Liver cirrhosis has been suggested as an independent risk factor for ICC in some case–control studies where liver inflammation was not included as a covariate [7,11,12]. We were not able to assess the risk of liver cirrhosis for ICC because of lack of this information in the blood-donor database, but patients with known liver cirrhosis were excluded in our study upon screening before blood donation, minimizing the effect of a potential confounding factor. In part, the presence of liver cirrhosis among ICC cases in the case–control studies might be a proxy of present or past liver inflammation. If so, ICC might be associated with liver inflammation in cohort studies using subjects in a pre-cirrhosis state, and with liver cirrhosis in case–control studies. The only case–control study that included both liver cirrhosis and inflammation as covariates demonstrated that ICC was significantly associated with the presence of inflammation but not with liver cirrhosis [13]. Further studies on this issue are necessary.

Our study has some potential limitations. First, information on the presence of established major risk factors for ICC, namely infection with liver fluke, intrahepatic biliary stones, fibropolycystic liver disease and primary sclerosing cholangitis, was not available in the blood-donor database, and we were not able to adjust the hazard ratios for them. However, liver fluke (*Clonorchis sinensis*) infection was already rare in Osaka and surrounding areas by the 1960s [29]. Also, patients with chronic liver or autoimmune diseases were excluded from blood donors upon their claim in the screening questionnaire. Therefore, influence from known confounding factors should be minimal in our study. Second, the number of ICC cases in our study was fairly limited because of the relatively low incidence of ICC, resulting in a wide confidence interval in the estimated incidence rates or relative risks. Our findings on the risk of viral infection and inflammation, however, were consistent with observations in past studies, and it should, at least in part, warrant the validity of our findings. Third, we may have underestimated the incidence rates of ICC in our study, because the number of person-years of observation described in the Results section was not adjusted by the number of subjects who might have moved out of the province or died, before the final date of follow-up. The estimated incidence rates, after adjustment using the revised values for these parameters, would be approximately 25% higher than the figures described earlier.

Table 3 Results of analytical studies on the association of intrahepatic cholangiocarcinoma (ICC) and hepatits B and C virus

Authors and year	Study area	Type of study	No. of ICC case	RR (95% CI) for HBV infection*	RR (95% CI) for HCV infection*
Shin *et al.* 1996 [30]	Pusan, Korea	Case–control, hospital- based	41	OR = 1.3 (0.3–5.3)	OR = 3.9 (0.9–17.1)
Donato *et al.* 2001 [14]	Italy	Case–control, hospital- based	26	OR = 2.7 (0.4–18.5)	OR = 9.7 (1.6–58.9)
Yamamoto *et al.* 2004 [13]	Osaka, Japan	Case–control, hospital- based	50	OR = 1.8 (0.3–10.1)	OR = 16.8 (5.7–50.0)
Shaib *et al.* 2005 [12]	TX, USA	Case–control, Medicare- beneficiaries	625	OR = 0.8 (0.1–5.9)	OR = 6.1 (4.3–8.6)
Welzel *et al.* 2007 [11]	USA	Case–control, population-based	535	–	OR = 5.4 (2.9–10.2)
Shaib *et al.* 2007 [9]	TX, USA	Case–control, hospital-based	83	OR = 28.6 (3.9–1268.1)†	OR = 7.9 (1.3–84.5)
Lee *et al.* 2008 [7]	Korea	Case–control, hospital-based	622	OR = 2.3 (1.6–3.3)	OR = 1.0 (0.5–1.9)
Zhou *et al.* 2008 [8]	Shanghai, China	Case–control, hospital-based	312	OR = 8.8 (5.9–13.1)	OR = 0.9 (0.3–3.1)
Lee *et al.* 2009 [6]	Taiwan	Case–control, hospital-based	160	OR = 5.0 (2.8–9.0)	OR = 2.7 (1.2–6.3)
El-Serag *et al.* 2009 [10]	USA	Cohort, veterans population	37	–	HR = 2.6 (1.3–5.0)
Tanaka *et al.* (present)	Osaka, Japan	Cohort, blood donor population	11	HR = 8.6 (1.3–55.2)	HR = 2.6 (0.3–27.7)

Note: Hepatitis B virus infection status was identified by the presence of HBs antigen except the study by Shaib *et al.* 2007. (–): not asssessed as a covariate. Cl, confidence interval; HBV, hepatitis B virus; HCV, hepatitis C virus; RR, relative risk of develping intrahepatic cholangiocarcinoma; OR, adjusted odds ratio by multiple logistic regression analysis; HR, adjusted hazard ratio by Cox proportional hazard regression analysis. *The original figures reported in each study were rounded up to the first decimal place. †HBV infection status verified by the antiHBc+ and HBsAg–; Odds ratio for HBsAg+/antiHBc– was shown to be 2.9 (95%CI: 0.1–236.9).

Lastly, coverage of the cancer registry in Osaka was not perfect, potentially reducing the estimated incidence of ICC. Nevertheless, we assume that the coverage for cancers with poor prognosis, like ICC, was sensitive enough because of the use of death certificate information. Also, the imperfect coverage of the registry should not affect the relative risk for HBV/HCV infection or liver inflammation because the registry coverage was independent of the presence of these risk factors.

In conclusion, our results suggest that HBV infection and liver inflammation are independently associated with ICC development. These findings as well as their association with liver cirrhosis and other potential risk factors need to be verified by further large cohort studies.

ACKNOWLEDGEMENT

This study was financially supported by a Grant-in-Aid for Cancer Research and a Grant-in-Aid for the Third Term Comprehensive 10-Year Strategy for Cancer Control from the Japanese Ministry of Health, Labor and Welfare, and by a Grant-in-Aid for Scientific Research (B) from the Japanese Ministry of Education, Culture, Sports, Science, and Technology. We thank Dr Hiroshi OHMAE, Institutes of Infectious Diseases, Tokyo Dr Kazuhito Katayama, Dr Katsuyuki Nakanishi, and Ms Yasue KOUTANI, Osaka Medical Center for Cancer and Cardiovascular Diseases, for their technical assistance.

STATEMENT OF INTERESTS

Author's declaration of personal interests: None of the authors claim potential conflict of interest that pertains to this manuscript.

Declaration of funding interests: This study was funded in part by a Grant-in-Aid for Cancer Research and a Grant-in-Aid for the Third Term Comprehensive 10-Year Strategy for Cancer Control from the Japanese Ministry of Health, Labor

and Welfare, and by a Grant-in-Aid for Scientific Research (B) from the Japanese Ministry of Education, Culture, Sports, Science, and Technology.

REFERENCES

1 Shaib Y, El Serag HB. The epidemiology of cholangiocarcinoma. *Semin Liver Dis* 2004; 24: 115–125.
2 Vauthey JN, Blumgart LH. Recent advances in the management of cholangiocarcinomas. *Semin Liver Dis* 1994; 14: 109–114.
3 Farley DR, Weaver AL, Nagorney DM. "Natural history" of unresected cholangiocarcinoma: patient outcome after noncurative intervention. *Mayo Clin Proc* 1995; 70: 425–429.
4 Khan SA, Thomas HC, Davidson BR, Taylor-Robinson SD. Cholangiocarcinoma *Lancet* 2005; 366: 1303–1314.
5 Tanaka M, Tsukuma H. Epidemiology of cholangiocarcinoma. *Nippon Rinsho (Japanese)*. 2009; 67(Suppl. 3): 278–282.
6 Lee CH, Chang CJ, Lin YJ. Viral hepatitis-associated intrahepatic cholangiocarcinoma shares common disease processes with hepatocellular carcinoma. *Br J Cancer* 2009; 100: 1765–1770.
7 Lee TY, Lee SS, Jung ST *et al.* Hepatitis B Virus Infection and Intrahepatic Cholangiocarcinoma in Korea: A Case-Control Study. *Am J Gastroenterol* 2008; 103: 1716–1720.
8 Zhou YM, Yin ZF, Yang JM *et al.* Risk factors for intrahepatic cholangiocarcinoma: a case–control study in China. *World J Gastroenterol* 2008; 14: 632–635.
9 Shaib YH, El-Serag HB, Nooka AK *et al.* Risk factors for intrahepatic and extrahepatic cholangiocarcinoma: a hospital-based case-control study. *Am J Gastroenterol* 2007; 102: 1016–1021.
10 El-Serag H, Engels E, Landgren O *et al.* Risk of hepatobiliary and pancreatic cancers after hepatitis C virus infection: a population-based study of U.S. veterans. *Hepatology* 2009; 49: 116–123.
11 Welzel TM, Graubard IB, El-Serag BH *et al.* Risk factors for intra-and extrahepatic cholangiocarcinoma in the United States: a population based case control study. *Clin Gastroenterol Hepatol* 2007; 5: 1221–1228.
12 Shaib YH, El-Serag HB, Davila JA, Morgan R, McGlyn KA. Risk factors of intrahepatic cholangiocarcinoma in the United States: a case-control study. *Gastroenterology* 2005; 128: 620–626.
13 Yamamoto S, Kubo S, Hai S *et al.* Hepatitis C virus infection as a likely etiology of intrahepatic cholangiocarcinoma. *Cancer Sci* 2004; 95: 592–595.
14 Donato F, Gelatti U, Tagger A *et al.* Intrahepatic cholangiocarcinoma and hepatitis C and B virus infection, alcohol intake, and hepatolithiasis: a case-control study in Italy. *Cancer Causes Control* 2001; 12: 959–964.
15 Tanaka H, Tsukuma H, Yamano H *et al.* Prospective study on the risk of hepatocellular carcinoma among hepatitis C virus positive blood donors focusing on demographic factors, alanine aminotransferase, level at donation and interaction with hepatitis B virus. *Int J Cancer* 2004; 112: 1075–1080.
16 Tanaka H, Tsukuma H, Hori Y *et al.* The risk of hepatitis C virus infection among blood donors in Osaka, Japan. *J Epidemiol* 1998; 8: 292–296.
17 Kotani K, Maekawa M, Kanno T. Reestimation of aspirate aminotransferase/alanine aminotransferase ratio based on JSCC consensus method. *Jpn J Gastro-enterology (Japanese)* 1994; 91: 154–161.
18 Watanabe J, Matsumoto C, Fujimura K *et al.* Predictive value of screening tests for persistent hepatitis C virus infection evidenced by viraemia. Japanese experience. *Vox Sang* 1993; 65: 199–203.
19 Ajiki W, Tsukuma H, Oshima A *et al.* Osaka prefecture. In: Parkin DM, Whelam SL, Ferlay J, Teppo L, Thomas DB, eds. Cancer Incidence in Five Continents, Vol. 8. Lyon: IARC, 2002: 264–265.
20 International Agency for Cancer Research. Cancer Incidence in Five Continents, vol. 8. Lyon: IARC Scientific Publication No. 155, 2002.
21 Tsukuma H, Tanaka H, Ajiki W. Liver cancer and its prevention. *Asian Pac J Cancer Prev*, 2005; 6: 244–250.
22 Kobayashi M, Ikeda K, Saitoh S *et al.* Incidence of primary cholangiocellular carcinoma of the liver in Japanese patients with hepatitis C virus-related cirrhosis. *Cancer* 2000; 88: 2471–2477.
23 Okuda K. The Liver Cancer Study Group of Japan: primary liver cancers in Japan. *Cancer* 1980; 45: 2663–2669.
24 Ikai I, Avii, Okazaki M *et al.* Report of the 17th Nationwide Follow-up Survey of Primary Liver Cancer in Japan. *Hepatol Res* 2007; 37: 676–691.
25 Perumal V, Wang J, Thuluvath P, Choti M, Torbenson M. Hepatitis C and hepatitis B nucleic acids are present in intrahepatic cholangiocarcinomas from the United States. *Hum Pathol* 2006; 37: 1211–1216.
26 Wang WL, Gu GY, Hu M. Expression and significance of HBV genes and their antigens in human primary intrahepatic cholangiocarcinoma. *World J Gastroenterol* 1998; 4: 392–396.
27 Zou SQ, Qu ZL, Li ZF, Wang X. Hepatitis B virus X gene induces human telomerase reverse transcriptase mRNA expression in cultured normal human cholangiocytes. *World J Gastroenterol* 2004; 10: 2259–2262.
28 Blechacz B, Gores JG. Cholangiocarcinoma. Advances in pathogenesis, diagnosis, and treatment *Hepatology* 2008; 48: 308–321.
29 Meguro Parasitological Museum. The distribution and epidemiology of liver flukes in Japan. *Prog Med Parasitol Jpn (Japanese)* 1962; 2: 359–361.
30 Shin HR, Lee CU, Park HJ *et al.* Hepatitis B and C virus, Clonorchis sinensis for the risk of liver cancer: a case control study in Pusan, Korea. *Int J Epidemiol* 1996; 25: 933–940.

VI. 肝癌の疫学

胆管細胞癌の疫学

Epidemiology of cholangiocarcinoma

田中政宏　津熊秀明

Key words：胆管細胞癌，肝内胆管癌，疫学，罹患率，リスク

はじめに

原発性肝癌は，国際疾病分類(ICD-10)では病理組織像の特性に基づき，肝細胞癌(C22.0)，胆管細胞癌(C22.1)，肝芽腫(C22.2)，肝血管肉腫(C22.3)，その他の肝の肉腫(C22.4)，その他の明示された肝の癌(混合型を含む)(C22.7)，詳細不明の肝の悪性腫瘍(C22.9)の7群に分類される．胆管細胞癌(肝内胆管癌)は，肝内胆管上皮に由来する癌腫で，肝門部に近い比較的太い胆管から末梢の細い胆管に至るまで，どの部位からでも発生しうる．なお左右の肝管および両者の合流部近傍に発生する胆管癌は，肝門部胆管癌とも呼ばれ，肝外胆管癌に分類される．胆管細胞癌は，原発性肝癌の中では肝細胞癌に次ぐ頻度である．

本稿では，はじめに我が国における胆管細胞癌の現況・動向について述べ，次いで胆管細胞癌の発生要因について，これまでの知見を総説的に述べる．

1. 我が国における胆管細胞癌発生の現況・動向

我が国の原発性肝癌の性別頻度分布を**表1**に示した．このうち人口動態死亡統計は厚生労働省大臣官房統計情報部による2000-04年の5年間の成績，罹患統計は，大阪府がん登録データによる2000-04年値，および，日本肝癌研究会による2002-03年の新規登録例(第17回全国原発性肝癌追跡調査報告[1])についての集計値である．

大阪府がん登録は，大阪府在住者に発生したすべてのがんを，府内の医療機関に依頼して届け出てもらい登録する制度であり，診断・治療・病理組織型などに関する一定の情報をデータベース化している．**表1**における同データにおいては，罹患年が2000-04年に診断された肝癌についてまず国際疾病分類腫瘍学形態学コード(ICD-OM第3版)による集計を行い，次にParkinらの国際比較研究のための組織型群の考え[2]に従いグループ化し，最終的にICD-10に基づく7つのサブカテゴリーに再構成した．

胆管細胞癌の占める割合は，死亡統計では男性6.0％，女性10.0％，大阪府がん登録では男性5.0％，女性8.5％，全国肝癌登録では男性3.7％，女性5.5％であった．ただし，それぞれのデータの解釈には留意が必要である．死亡統計では，原死因が'肝癌'とされた場合C22.0とコード化されるため，胆管細胞癌の比率が過小評価される可能性のあること，全国肝癌追跡調査では登録症例の代表性に制約があると推測されること，大阪府がん登録では登録症例の代表性には大きな問題はないが，病理組織型報告例の割合が低いことを保留しておく．ともあれ我

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表1　原発性肝癌の組織型分布（臨床診断または組織診断による）

肝および肝内胆管の悪性新生物		人口動態死亡統計 厚生労働省統計情報部 2000–04年死亡				地域がん登録資料 大阪府がん登録 2000–04年罹患*				全国臓器別がん登録 日本肝癌研究会** 2002–03年新規登録症例			
		男性	%	女性	%	男性	%	女性	%	男性	%	女性	%
C22		117,810		53,718		13,054		5,784		13,017		5,196	
C22.0	肝細胞癌	109,888	93.9	47,785	89.8	11,070	94.7	4,606	91.1	12,341	94.8	4,818	92.7
C22.1	胆管細胞癌	6,979	6.0	5,301	10.0	583	5.0	431	8.5	485	3.7	285	5.5
C22.2	肝芽腫	37	0.0	29	0.1	10	0.1	6	0.1	8	0.1	4	0.1
C22.3	肝血管肉腫	41	0.0	28	0.1	2	0.0	4	0.1	11	0.1	8	0.2
C22.4	その他の肝の肉腫	47	0.0	28	0.1	13	0.1	6	0.1				
C22.7	その他の明示された肝の癌	50	0.0	22	0.0	8	0.1	3	0.1	172	1.3	81	1.6
総計（C22.9：詳細不明の肝の悪性腫瘍等を除く）		117,042	100.0	53,193	100.0	11,686	100.0	5,056	100.0	13,017	100.0	5,196	100.0

いずれの報告も臨床診断のみによる症例を含む．
*2000–04年の総報告数18,838例から，詳細不明の肝の悪性腫瘍（C22.9）（2,030例）およびParkinらの分類に含まれない組織型のもの（66例）を除く．
**C22.1にはcholangiocellular carcinomaだけでなくcystadenocarcinomaを含めた．C22.7にはmixed carcinomaとothersを計上した．

が国では胆管細胞癌が原発性肝癌の4–10％程度を占め，その割合は男性より女性で高いと推測される．

表2では大阪府がん登録資料に基づき年齢階級別の病理組織型分布を示した．肝癌の大多数は45歳以上に発生しており，またこの年齢階級以上では，肝細胞癌と胆管細胞癌の2つの組織型で肝癌全体の99％以上を占めていた．

次に，以上の肝癌病理組織型分布を考慮に入れ，大阪府における1980–84年，1990–94年，2000–04年の胆管細胞癌および肝細胞癌の年齢階級別罹患率を推計し，**図1**に示した．それぞれの年齢階級におけるC22.9（詳細不明）の報告分は，C22.9を除いた‘C22.0–22.4およびC22.7全体’におけるそれぞれの割合で按分して加えることにより，各10歳年齢階級の肝細胞癌と胆管細胞癌の罹患数を推計した．肝細胞癌罹患率の増加には，いずれの年齢階級でも近年頭打ちの傾向がみられたが，胆管細胞癌においては1980年代以降いずれの年齢階級においても罹患率は漸増しており，2000年代に入ってもその傾向は続いていた．また，いずれの年代においても高齢になるほど罹患率は高くなる傾向がみられた．

この図にみられる傾向と同様に，世界的には胆管細胞癌の罹患率および死亡率が近年増加しているとの報告が複数あり[3–5]，この現象の原因としては，肝癌の報告方法の変更または改善，診断技術の向上，そして罹患率の真の増加などが考えられる．そして，この増加が複数の国・地域でみられること，増加割合に性差がみられること，報告例中の早期がんの割合に変化がみられないことなどの理由から罹患率は真に増加しているとの主張もある[6]．この真偽については，胆管細胞癌のリスク要因も考慮した今後の研究が待たれる．

2. 胆管細胞癌のリスク要因

胆管細胞癌のリスクとされている要因には，肝吸虫の寄生，原発性硬化性胆管炎，肝臓結石，胆管枝の奇形，化学物質，ウイルス性肝炎などがある[6]．これらの要因の多くは胆管上皮の慢

表2 年齢階級別にみた肝癌の組織型分布（大阪府がん登録，2000-04年）

年齢階級	肝細胞癌 (C22.0)	胆管細胞癌 (C22.1)	肝芽腫 (C22.2)	肝血管肉腫 (C22.3)	その他の肝の肉腫 (C22.4)	その他の明示された肝の癌 (C22.7)	総　計
0-14	2 (10.0)	0 (0.0)	16 (80.0)	0 (0.0)	2 (10.0)	0 (0.0)	20 (100)
15-34	42 (95.5)	1 (2.3)	0 (0.0)	0 (0.0)	1 (2.3)	0 (0.0)	44 (100)
35-44	148 (90.2)	16 (9.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	164 (100)
45-54	1,069 (93.0)	79 (6.9)	0 (0.0)	0 (0.0)	1 (0.1)	1 (0.1)	1,150 (100)
55-64	3,272 (93.2)	229 (6.5)	0 (0.0)	4 (0.1)	3 (0.1)	2 (0.1)	3,510 (100)
65-74	7,028 (94.9)	360 (4.9)	0 (0.0)	0 (0.0)	10 (0.1)	5 (0.1)	7,403 (100)
75-	4,110 (92.4)	329 (7.4)	0 (0.0)	2 (0.0)	2 (0.0)	3 (0.1)	4,446 (100)
総計	15,671 (93.6)	1,014 (6.1)	16 (0.1)	6 (0.0)	19 (0.1)	11 (0.1)	16,737 (100)

ICD-10への分類：国際疾病分類腫瘍学形態学コード（ICD-OM第3版）による集計を行った後，Parkinらの国際比較研究のための組織型群の考えに従いグループ化した．

2000-04年の総報告数18,838例から，詳細不明の肝の悪性腫瘍（C22.9）（2,030例），C22.0における年齢不明例（5例）およびParkinらの分類に含まれない組織型のもの（66例）を除いた．

（　）内の数値は％．

性炎症と関連すると考えられる．

世界的にみると，肝吸虫症は胆管細胞癌の地域集積と密接に関連しており[7]，とりわけ，タイ，ラオス，カンボジアで流行のみられる*Opisthorchis viverrini*と，中国・台湾，韓国，ベトナムで流行のみられる*Clonorchis sinensis*との関連が注目されている．タイ東北部，中国，韓国で認められるように，肝吸虫は淡水魚の生食を通してヒトに感染し，成虫は主として肝内胆管に寄生する．

肝臓結石および原発性硬化性胆管炎は，肝吸虫症と同じく慢性胆管炎の原因となり，胆管の慢性炎症が過形成性変化，異型性変化へと移行する過程で，胆管細胞癌の発生と密接に関与すると考えられている[6]．肝臓結石は胆道の細菌感染と胆汁のうっ滞とが関連して発生し，我が国を含め極東地域の胆管細胞癌の前駆性病変になっている場合が多い[6]．原発性硬化性胆管炎は，とりわけ西欧諸国で胆管細胞癌の前駆性病変として考えられており[6]，原発性硬化性胆管炎の8-40％程度に胆管細胞癌の合併がみられる[6]．また，潰瘍性大腸炎患者に胆管細胞癌が併発する例も知られている．胆管枝の奇形などによる胆管嚢胞性拡張も結果的に慢性胆管炎を引き起こし，胆管細胞癌の危険因子になると考えられている．

胆管細胞癌の原因となりうる化学物質としては，放射性造影剤として使われたトロトラスト，ダイオキシン，ニトロサミン，喫煙に関連する物質，アルコール摂取などが報告されている[6]．

胆管細胞癌のほとんどは非硬変肝から発生するが，ウイルス性，アルコール性などの非胆汁性肝硬変に発生する胆管細胞癌も報告されている[8]．Kobayashiら[9]は，C型肝炎ウイルスによる肝硬変を有する日本人600人を平均7.2年間追跡調査し，11人に胆管細胞癌，3人に肝細胞癌

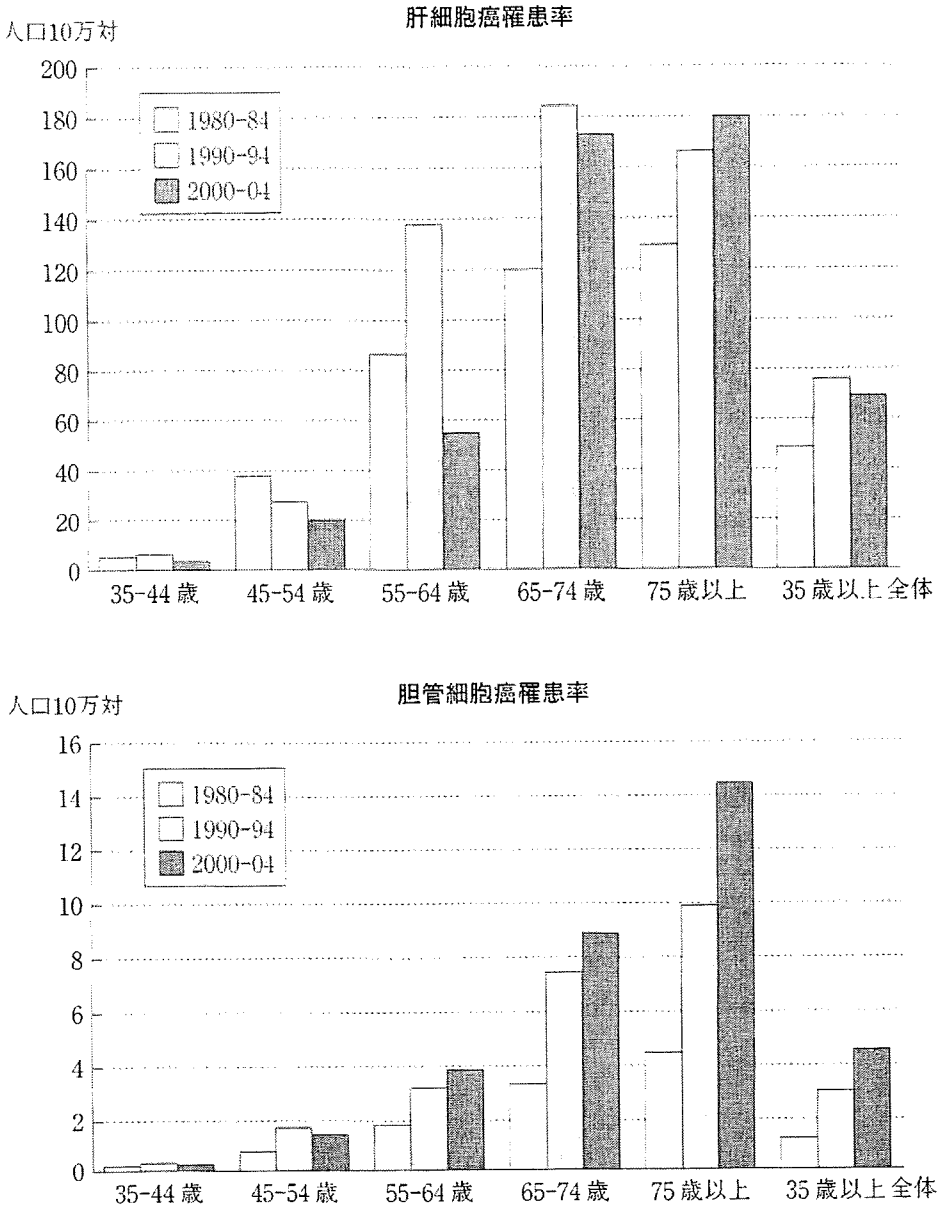

図1 肝細胞癌，胆管細胞癌の年齢別，年代別罹患率の推移(人口10万対)
(大阪府がん登録データより)
それぞれの年齢階級における詳細不明例(C22.9)の報告分は，C22.9を除いた'C22.0-22.4およびC22.7全体'におけるそれぞれの割合で按分して加えることにより，各10歳年齢階級の肝細胞癌と胆管細胞癌の罹患数を推計した．

と胆管細胞癌の混合型，206人に肝細胞癌の発生を認め，この群からの胆管細胞癌発生率が日本人一般人口からの発生率と比べ極めて高いと報じた(なおこの報告では，罹患率を全国肝癌追跡調査を基に推計している点で留意が必要である)．また，近年ウイルス肝炎と胆管細胞癌の関係を示唆する報告も複数みられる．大阪，イタリア，米国からはC型肝炎ウイルス感染によって胆管細胞癌罹患リスクが6-9倍程度高くなること[8,10,11]が報告されている．肝細胞と胆管細胞は同じ前駆細胞をもつこと，またC型肝炎ウイルスのRNAが胆管細胞癌組織に確認されたとの報告などもこの因果関係を示唆するが，正確な発癌のメカニズムは今後の課題である．また韓国からは，B型肝炎ウイルス感染により胆管細胞癌の罹患リスクが2倍程度になること

が報告されている[12]. 1980年代後半から90年代まで, 我が国ではC型肝炎による肝癌が著明に増加した. その大多数は肝細胞癌によるものであるが, このことが大阪がん登録データで示唆された胆管細胞癌罹患率の上昇とどのような関連があるのかにつき, 今後の検討が必要である.

■文　献

1) 日本肝癌研究会追跡調査委員会：第17回原発性肝癌追跡調査報告(2002-2003). 肝臓 **48**: 117-140, 2007.
2) Parkin DM, et al: Histological Groups for Comparative Studies, International Agency for Research on Cancer, Lyon, 1998.
3) Taylor-Robinson SD, et al: Increase in mortality rates from intrahepatic cholangiocarcinoma in England and Wales 1968-1998. Gut **48**: 816-820, 2001.
4) Patel T: Worldwide trends in mortality from biliary tract malignancies. BMC Cancer **2**: 10, 2002.
5) Shaib YH, et al: Rising incidence of intrahepatic cholangiocarcinoma in the United States: a true increase? J Hepatol **40**: 472-477, 2004.
6) Khan S: Cholangiocarcinoma. Lancet **366**: 1303-1314, 2005.
7) Vatanasapt V, et al: Liver flukes and liver cancer. In: Cancer Surveys, Infections and Human Cancer Vol. 33 (ed by Tooze J), p313-335, Cold Spring Harbor Laboratory Press, New York, 1999.
8) Shaib YH, et al: Risk factors of intrahepatic cholangiocarcinoma in the United States: a case-control study. Gastroenterology **128**: 620-626, 2005.
9) Kobayashi M, et al: Incidence of primary cholangiocellular carcinoma of the liver in Japanese patients with hepatitis C virus-related cirrhosis. Cancer **88**: 2471-2477, 2000.
10) Yamamoto S, et al: Hepatitis C virus infection as a likely etiology of intrahepatic cholangiocarcinoma. Cancer Sci **95**: 592-595, 2004.
11) Donato F, et al: Intrahepatic cholangiocarcinoma and hepatitis C and B virus infection, alcohol intake, and hepatolithiasis: a case-control study in Italy. Cancer Causes Control **12**: 959-964, 2001.
12) Lee TY, et al: Hepatitis B virus infection and intrahepatic cholangiocarcinoma in Korea: a case-control study. Am J Gastroenterol **103**: 1716-1720, 2008.

Mutation Research 678 (2009) 38–42

Contents lists available at ScienceDirect

Mutation Research/Genetic Toxicology and Environmental Mutagenesis

journal homepage: www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

Induction of SCEs in CHL cells by dichlorobiphenyl derivative water pollutants, 2-phenylbenzotriazole (PBTA) congeners and river water concentrates

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ARTICLE INFO

Article history:
Received 4 March 2009
Received in revised form 10 June 2009
Accepted 13 June 2009
Available online 21 June 2009

Keywords:
Sister chromatid exchange (SCE)
Chinese hamster lung (CHL) cells
Ames test
3,3′-Dichlorobenzidine (DCB)
4,4′-Diamino-3,3′-dichloro-5-nitrobiphenyl (5-nitro-DCB)
PBTA-1
PBTA-2
PBTA-6

ABSTRACT

We recently identified dichlorobiphenyl (DCB) derivatives and 2-phenylbenzotriazole (PBTA) congeners as major mutagenic constituents of the waters of the Waka River and the Yodo River system in Japan, respectively. In this study we examined sister chromatid exchange (SCE) induction by two dichlorobiphenyl derivatives, 3,3′-dichlorobenzidine (DCB, 4,4′-diamino-3,3′-dichlorobiphenyl) and 4,4′-diamino-3,3′-dichloro-5-nitrobiphenyl (5-nitro-DCB); three PBTA congeners, 2-[2-(acetylamino)-4-[bis(2-methoxyethyl)amino]-5-methoxyphenyl]-5-amino-7-bromo-4-chloro-2*H*-benzotriazole (PBTA-1), 2-[2-(acetylamino)-4-[*N*-(2-cyanoethyl)ethylamino]-5-methoxyphenyl]-5-amino-7-bromo-4-chloro-2*H*-benzotriazole (PBTA-2), and 2-[2-(acetylamino)amino]-4-[bis(2-hydroxyethyl)amino]-5-methoxyphenyl]-5-amino-7-bromo-4-chloro-2*H*-benzotriazole (PBTA-6); and water concentrates from the Waka River in Chinese hamster lung (CHL) cells. Concentration-dependent induction of SCE was found for all DCBs and PBTAs examined in the presence of S9 mix, and statistically significant increases of SCEs were detected at 2 μg per ml of medium or higher concentrations. SCE induction of MeIQx was examined to compare genotoxic activities of these water pollutants. According to the results, a ranking of the SCE-inducing potency of these compounds is the following: 5-nitro-DCB ≈ MeIQx > PBTA6 > PBTA-1 ≈ PBTA-2 > DCB.

Water samples collected at a site at the Waka River showed concentration-related increases in SCEs at 6.25–18.75 ml-equivalent of river water per ml of medium with S9 mix. The concentrations of 5-nitro-DCB and DCB in the river water samples were from 2.5 to 19.4 ng/l and from 4100 to 18,900 ng/l, respectively. However, these chemicals showed only small contribution to SCE induction by the Waka River water.

1. Introduction

Genotoxic compounds are detected in many surface waters in the world. These compounds are often released directly from industrial discharges as a result of insufficient treatment of wastewater or unintentional formation in the environment after discharge of effluents [1–5]. In the previous studies, we found two novel chemical classes, dichlorobiphenyl derivatives and 2-phenylbenzotriazole (PBTA) congeners, as major mutagenic constituents in the water of rivers flowing through several industrial areas in Japan [6–20].

Among dichlorobiphenyl derivatives, 3,3′-dichlorobenzidine (DCB, 4,4′-diamino-3,3′-dichlorobiphenyl), 4,4′-diamino-3,3′-dichloro-5-nitrobiphenyl (5-nitro-DCB), and so forth were identified as major mutagens in the water of the Waka River flowing through an industrial area in Wakayama, where a number of large chemical plants are found [6–9]. 5-Nitro-DCB is a novel chemical and is presumed to be formed unintentionally by the process of wastewater treatment of drainage water containing DCB discharged from chemical plants [6]. DCB is a raw material in the manufacture of polymers and dye intermediates, and there are large-scale chemical plants producing DCB in this industrial area. 5-Nitro-DCB is highly mutagenic in the Ames assay using *Salmonella typhimurium* YG1024, which is an *O*-acetyltransferase-overproducing derivative of TA98, with S9 mix, and its activity was ~7 times higher than that of DCB.

5-Nitro-DCB was detected in river water concentrates at the maximum level of 6.9 μg/g of blue rayon. DCB was also detected in the concentrates at 13.2–104 μg/g of blue rayon. The percent contributions of 5-nitro-DCB and DCB to the mutagenicity of the water concentrates in YG1024 with S9 mix were 11% and 28%, respectively,

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doi:10.1016/j.mrgentox.2009.06.003