Epidemiological Studies on the Distribution and Determinants of Biliary Tract Cancer

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Abstract

With the help of my colleagues, I have been conducting epidemiological studies on biliary tract cancer (BTC), including gallbladder cancer (GBC) and extrahepatic bile duct cancer (BDC), in Japan and Chile for about 19 years. Clustered areas with high mortality rates, especially for female GBC were found to correspond with places or prefectures in Japan that were famous for rice production. The roles of known risk factors, such as gallstones and cholecystitis, were examined, but no single factor was implicated in the high mortality rates for GBC in these areas. A working hypothesis, called the “rice production hypothesis” was formulated; this initial hypothesis was replaced by a new multifactorial causation hypothesis: GBC is more likely to occur in individuals with a genetic susceptibility and a past history of gallstones or cholecystitis who are exposed to geographically specific environmental factors, such as agricultural chemicals. On the basis of various analytical studies, it is concluded that a certain agricultural chemical was responsible for the occurrence of GBC. At the time of writing, no evidence has been obtained to disprove our hypothesis.

We have also conducted international collaborative studies in Chile, which has the highest mortality rate for GBC in the world. Bile from Chileans was found to have a higher mutagenic activity than that from Japanese subjects; Chileans with a history of constipation or a habit of consuming red chilli pepper had a high risk of developing GBC, if they also had gallstone(s). The presence of a regional difference in p53 mutagenesis was also observed.

Key words: epidemiology, biliary tract cancer, gallbladder cancer, bile duct cancer, Japan, Chile

Introduction

With the help of my colleagues in Japan and Chile, I have been conducting epidemiological studies on biliary tract cancer (BTC), including gallbladder cancer (GBC) and extrahepatic bile duct cancer (BDC), for about 19 years. My main reason for conducting such epidemiological studies goes back to 1980, when I graduated from the University of Texas School of Public Health. During an epidemiology course, I became interested in geographically clustered diseases, especially from the point of view of genetic and environmental interactions. Upon my return to Japan, I decided to investigate the distribution and determinants of geographically clustered diseases. During my initial literature survey on demographic data in Japan, I discovered on study (1) reporting a characteristic clustering pattern of BTC in Niigata, where I have lived for about 40 years at the time of this presentation. As an epidemiologist, I was very surprised by the report (1) and immediately began to study the distribution and determinants of BTC.

In 1984, I was fortunate to participate in a research project sponsored by a Grant-In-Aid from the Ministry of Health and Welfare and began epidemiological studies on BTC as part of the Niigata Cancer Research Project, conducted by the late Drs. Norio Abe and Sadahiko Akai (previous directors of Niigata Cancer Center Hospital). In 1991, I was able to extend my research to a global level and was given the opportunity to study in Chile, thanks to a Grant-In-Aid from the Ministry of Education, Science and Culture.

The present paper summarizes the findings that I and my colleagues have obtained in Japan and Chile over the last 19 years and provides basic epidemiological information on BTC (2).
prefectures with the lowest scores were Nara, Tottori and Okinawa. In females, the highest SMRs were obtained in Niigata, Aomori and Yamagata, while the lowest SMRs were obtained in Hiroshima, Kochi and Shimane. Thus, a cluster of prefectures in the northeastern part of Japan was found to exhibit the highest SMRs in both genders. On the other hand, prefectures in the southwestern part of Japan tended to have low SMRs (3, 4). These findings were consistent with those of a previous study by Tominaga et al. (5).

Mortality data for BTC cases between 1979 and 1983 in Niigata Prefecture were obtained from death certificates filed at the vital statistics office, and the SMRs for both genders were analyzed for 36 cities and counties and then compared with that of the Japanese population in 1985 (6, 7). Clustered areas with high SMRs, especially for females, were found to correspond to the Echigo plains, which are famous for rice production. However, a few exceptions with low SMRs (the cities of Murakami, Gosen and Kamo) were noted. In a later study, we confirmed that this characteristic BTC clustering pattern was due to the occurrence of GBC, rather than BDC, by analyzing recent demographic data (8). We subsequently focused our attention on the etiology of GBC, rather than BDC, to discover the determinant(s) of BTC in Niigata in addition to Japan as a whole.

2) Determinants of GBC distribution

1. Known risk factors—First, we examined the role of known risk factors to see whether they were applicable to the determination of GBC in Niigata and Japan. For convenience, we summarize epidemiological characteristics of BTC, including GBC and BDC in Table 1 (9). As shown in the numbers from 6 to 11 of Table 1, risk factors such as gallstones, cholecystitis, typhoid fever, liver flukes (*Clonorchis sinensis*), ulcerative colitis, abnormal arrangement of the pancreatoco-biliary duct and a gastrectomy were examined to clarify whether the distribution of BTC, especially GBC was explained by these determinants. The possible involvement of industrial chemicals, such as asbestos and trichloroethylene was also examined. However, no single factor was capable of explaining the characteristic clustering pattern of GBC.

2. Rice production hypothesis—We next formulated a working hypothesis based on the characteristic geographic distribution of the high SMRs for GBC in Niigata and Japan. We proposed that the areas with high SMR values corresponded with those involving rice production. Consequently, we began to search for a link between them under a rice production and GBC mortality (10).

First, possible environmental factors were examined, including environmental contamination with agricultural chemicals, possible mutagenic or carcinogenic substances in the foods consumed in rice producing areas, and trace elements originating from paddy fields. We performed various analytical studies to test our rice production hypothesis (10).

a. Agricultural chemicals To investigate the possible relation between environmental contamination with agricultural chemicals and the BTC mortality rate, we conducted an ecological correlation study. The main reason we decided to first investigate the role of agricultural chemicals was that in 1986, Japan accounted for about 62.1% of the total amount of herbicide used among eight rice-producing countries throughout the world, even though the area of rice production in Japan accounts for only 1.6% of the area used for rice production throughout the world (11).

The officially reported amounts of agricultural chemical products distributed to 47 prefectures (12) were divided by the total prefectural area and the value, in kg/km² or /km², was designated as the environmental pollution index (EPI). The EPIs of each chemical product from 1962 to 1975 were compared with the SMRs of BTC in 1985 using Pearson’s correlation coefficient analysis. Of about 500 chemicals examined, the EPIs of MCPA-E, MCPB-E, CNP (Chlornitrofen), NIP (Nitrofen) and PCNB were frequently correlated with the SMRs (10, 13). These chemicals were characterized as either phenoxy herbicides (MCPA-E, MCPB-E), diphenylether herbicides (CNP, NIP) or phenol (PCNB) and all were reportedly contaminated with dioxins. Since the present findings were based on an ecological correlation analysis, the possible existence of ecological fallacy must be taken into consideration. To further clarify this finding, studies on the level of contamination, the route of human exposure, the carcinogenicity of these chemicals and other confounding factors should be conducted.

b. Foods consumed in rice producing areas The populations of prefectures with high SMRs for BTC tend to have a less-westernized diet that is lower in fat and protein than that of other areas in Japan (5, 7). However, it appears unlikely that the less-westernized diets of populations in rural areas play an important role in GBC mortality.

Table 1  Epidemiological Characteristics of Biliary Tract Cancer (BTC), Including Gallbladder Cancer (GBC) and Extrahepatic Bile Duct Cancer (BDC)

| Notes: | As one of the factors, agricultural chemicals reported by us is included in this table. |
| Source: | Ref. (9) |
role in the epidemiology of BTC, because food habits are rapidly becoming westernized, even in rural areas and this evidence would contradict the increase in BTC mortality rates that has been seen in Japan.

Regarding the role of food consumption, Kato et al. (14) reported in a case-control study of GBC and BDC that the consumption of oily foods and under-eating were high risk factors. However, the biological mechanism behind these risks was unclear. They also reported the intriguing findings (14) that a past history of gallstones or cholecystitis was a definite risk factor for both GBC and BDC, even in Niigata. If these diseases had been indigenous to the geographically clustered areas of high SMRs for BTC in Niigata, their findings could have paved the way to the discovery of the etiology of this phenomenon. Unfortunately, no historical evidence of such endemics was found in Niigata. To fill the gap between the case-control and ecological correlation studies, a multifactorial causation hypothesis was proposed, as will be described later.

c. Geological characteristics—River sediment from 19 sampling points near drinking water sources was collected and the distribution of 4 trace elements (Se, Mo, As and Sn) was analyzed. The ecological correlation between the level of trace elements and the SMRs of BTC in the cities and towns where the river sediment samples were obtained was then examined. No significant correlations were found (15).

3. Multifactorial causation hypothesis—When known risk factors of GBC, such as gallstones and cholecystitis, were examined, no single factor was capable of explaining the distribution of GBC in Niigata. In the above-mentioned case-control study, however, a past history of biliary disease, such as gallstones and cholecystitis, was definitely a risk factor (14). To explain the discrepancy in these findings, we decided to continue our studies. Before formulating a new hypothesis, we obtained new findings concerning a genetic susceptibility to GBC that was related to the histocompatibility antigen (HLA). Thirty-one patients with GBC and 32 control subjects were typed using antisera against 12 HLA-A, 3 HLA-B, 7 HLA-C and 13 HLA-DR antigens (16). The frequency of DR4 was found to be significantly higher in the GBC patients than in the control patients (61.3% vs 28.1%). Based on these findings, we generated a new multifactorial causation hypothesis. GBC may occur when individuals with a genetic susceptibility and past history of gallstones or cholecystitis are exposed to geographically specific environmental factors, such as agricultural chemicals.

4. Testing the new hypothesis—In the section on the distribution of SMRs for BTC, we mentioned that the areas with high SMR values (especially in females) in Niigata corresponded to the Echigo plains, with a few exceptions; the cities of Murakami, Gosen and Kamo all showed low SMRs. We attempted to discover which factor(s) were responsible for the characteristic distribution. We finally noted a difference in the sources of drinking water; cities with high SMRs obtained their water from the Shinano or Agano rivers, while those with low SMRs obtained their water from underground, mountain reservoirs or small rivers originating from mountain areas. Thus, a water-borne route via the contaminated Shinano or Agano rivers was highly suspected (7).

Based on the ecological correlation analysis, the agricultural chemicals under suspicion are phenoxy herbicides (MCPA-E, MCPB-E), diphenylether herbicides (CNP, NIP), phenol (PCNB) and dioxins. MCPA-E and MCPB-E were not detected in 1992, probably because of the reduction in their use. Concerning the carcinogenicity of these phenoxy herbicides, a relation with soft tissue sarcoma and non-Hodgkin’s lymphoma was reported (17). As far as we know, however, no epidemiological evidence of endemic soft-tissue sarcoma or non-Hodgkin’s lymphoma has been observed among the residents of Niigata. The use of NIP was banned in 1982; accordingly, an epidemiological analysis was not possible at the time of analysis in 1992. According to a review by Quest et al. (18), NIP as well as other diphenylether herbicides are carcinogenic. CNP was not reviewed at that time, probably because this chemical was only being used in Japanese paddy fields and the reviewers did not pay attention much to it. Since PCNB is a fungicide that is used for vegetables, rather than rice, and the water-borne exposure route for this chemical is negligible, we excluded it from further consideration. Dioxins are also unlikely to be involved in the occurrence of GBC because they are insoluble in water. However, a mechanism whereby dioxins act as a promoter (19), thereby leading to an environment that is favorable to the formation of GBC, cannot be discounted.

Regarding the role of CNP in the occurrence of BTC, especially in females with GBC, the CNP concentrations in faucet water collected from Niigata City (high SMR) and Joetsu City (low SMR) were examined in 1992 using ECD-gaschromatography. The maximum CNP levels were 554.24 and 2.09 ng/l in Niigata and Joetsu, respectively, on the same day in May. Details of the analysis are reported elsewhere (20, 21). In 1993, we analyzed the CNP concentration in faucet water from the cities of Murakami, Gosen and Kamo (areas with a low SMR for BTC) that are located in the midst of geographically clustered area with high SMRs in the Echigo plains. As shown in Table 2, the concentration levels of CNP in the cities of Niigata, Murakami, Gosen and Kamo were

<table>
<thead>
<tr>
<th>City</th>
<th>SMR</th>
<th>Male</th>
<th>Female</th>
<th>Source of sampling point</th>
<th>CNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niigata</td>
<td>51</td>
<td>190.08*</td>
<td>153.72*</td>
<td>Shinano river</td>
<td>76</td>
</tr>
<tr>
<td>Murakami</td>
<td>51</td>
<td>162.51</td>
<td>92.45</td>
<td>Groundwater</td>
<td>3</td>
</tr>
<tr>
<td>Gosen</td>
<td>51</td>
<td>128.63</td>
<td>128.63</td>
<td>Groundwater</td>
<td>6</td>
</tr>
<tr>
<td>Kamo</td>
<td>51</td>
<td>116.84</td>
<td>119.70</td>
<td>Kamo river</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Shinano river*</td>
<td>51</td>
</tr>
</tbody>
</table>

* Concentrations are expressed as ng/l (ppt); Quantity limits are less than 0.25 ng/l.
* Samples were collected on May 16–17, 1993.
* Standardized mortality ratio of gallbladder cancer (1981–1990); Population of Japan, 1985 was used as a standard; * p<0.001.
* Data resources are from Yamamoto.
* The use of faucet water from the Shinano river was started in 1973.
76.47, 3.34, 6.09 and 8.76 ng/l, respectively, on May 16–17, 1993. The levels of CNP concentrations were well correlated with the SMRs. On the basis of these analytical studies, it was concluded that further studies to clarify the distribution and occurrence of GBC were needed.

Recently, we obtained evidence suggesting another genetic susceptibility to GBC. We studied the relation between cytochrome P4501A1 (CYP1A1) gene polymorphisms and the risk of GBC (22). The main reason for performing this study was to obtain further information on the ecological correlation between the prevalence of GBC in rice producing areas of Japan and the use of agricultural chemicals, especially diphenylether herbicides such as CNP, NIP and its contaminant, dioxin. We investigated the frequency of the Mspl and Ile-Val polymorphisms of the CYP1A1 gene in 52 GBC cases and 104 healthy controls. As shown in Table 3, the frequencies of genotypes C and Ile/Val were significantly higher in the female patients with GBC than in the controls, suggesting a high genetic susceptibility to the development of GBC in females with this genotype.

In 1994, we proposed that a combination of genetic susceptibility (e.g. HLA-DR4), a past history of gallstones or cholecystitis, and environmental factors such as diphenylether herbicides (e.g. CNP, NIP) were responsible for the geographical clustering of BTC, especially in females with GBC, in Niigata based on the above findings (with the exception of the CYP1A1 gene polymorphism data).

After the presentation of a series of our findings, a committee of the Ministry of Health and Welfare concluded in 1994 that the use of CNP should be banned, since our findings strongly pointed towards a possible association between CNP use and the occurrence of BTC, especially GBC (23).

5. Changes in SMRs for BTC after the CNP ban in 1994—CNP was banned from use in 1994, but the amount of CNP in use had already been decreasing since 1975 at the time of this administrative decision. We, therefore, considered the effects of CNP exposure on the occurrence of GBC after 1975, rather than after the official ban in 1994. We have examined changes in the mortality data for BTC up until 1999. Figure 1 shows the age-adjusted mortality rates for BTC in Japan (24). The standard population is based on a model Japanese population in 1985. As shown in the figure, the mortality rate for BTC in Japanese females has been decreasing since the late 1980s. A birth cohort analysis also demonstrated a decrease in age-specific mortality rates among younger birth cohorts (Fig. 2). Figure 3 shows the age-specific mortality rates for birth cohorts of women born after 1938. The most probable explanation is either a change in the effects of risk factors, such as the agricultural chemicals discussed above, and/or the development of abdominal ultrasound sonography and laparoscopic chole-

Table 3 Association between GBC risk and CYP1A1 polymorphisms

<table>
<thead>
<tr>
<th>Mspl polymorphism</th>
<th>Ile-Val polymorphism</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Females</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>(0.53–3.45)</td>
</tr>
<tr>
<td>Males</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>(0.24–2.46)</td>
</tr>
<tr>
<td>Total</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>(0.52–2.23)</td>
</tr>
</tbody>
</table>

Odds ratio (95% confidence interval).

\(^a\) Significantly different from genotype A (p<0.05).

\(^b\) Significantly different from genotype Ile/Ile (p<0.05).
cystectomy. Future trends of demographic mortality rates as well as changes in the effects on birth cohort should continue to be examined. In addition, further research should examine the disappearance of the geographic clustering of GBC in Niigata and Japan.

**Epidemiological studies in Chile**

1) **International distribution of SMRs for BTC**

Mortality data for BTC in 39 countries were obtained from WHO for the period between 1981 and 1986. The SMRs were calculated based on the male and female period standard populations and the standard deaths by summing the age group-specific populations and death numbers in 39 countries (25). Among these 39 countries, Chile had the highest SMRs for both genders, while Japan had the second highest SMR for males and the fifth highest for females. These findings prompted us to conduct international collaborative studies between Chile and Japan.

In 1991, we had the opportunity to extend our research as part of the International Scientific Research Program sponsored by the Ministry of Education, Science and Culture. Since then, we have conducted three kinds of studies examining the mutagenic activity of gallbladder bile from cholecystectomized patients with gallstones, a case-control study of GBC, and a p53 analysis in GBC patients.

2) **Determinants of GBC distribution**

According to a study by Serra et al. (26), one of my colleagues, GBC accounts for approximately 90% of BTC cases in Chile. We, therefore, used the term GBC, instead of BTC, in our research in Chile.

1. Mutagenic activity of cholecystectomized gallbladder bile—

Bile samples were collected from female patients with gallstones, who underwent operations in Chile or Japan from Niigata, which had a high SMR, and Kochi, which had a low SMR. Although the details of the materials and methods were described elsewhere (27), these points will be briefly reviewed. The collected samples were treated using the blue rayon technique to detect mutagenic substances, especially heterocyclic amines. Extracts were then tested for mutagenicity using the pre-incubation method and *Salmonella typhimurium* TA98 in the presence of S9 mix. Of the 24 samples obtained from Chilean patients, 20 (83.3%) exhibited signs of mutagenicity. In Japan, 21 (80.8%) of 26 samples obtained from patients living in Niigata and 5 (19.2%) of 26 samples obtained from patients living in Kochi showed signs of mutagenicity. The positive rate in Chile was thus almost equal to that in Niigata but far greater than that for Kochi, Japan. However, the average number of revertant colonies was different for each group: 128±92 (mean±SD) for the Chilean samples, 62±14 for the samples from Niigata and 66±13 for the samples from Kochi. In summary, the Chilean bile had a higher mutagenic activity level than the Japanese bile. In a Japanese study, we recently discovered that the presence of free fatty acids can inhibit the mutagenic activity of blue rayon-treated bile samples (28, 29). We proceeded to perform a comparative study between Japan and Chile.

2. Case-control study—Based on the multifactorial causation hypothesis, we decided to look for GBC risk factor(s) associated with the presence of gallstones. Ninety GBC cases, all of whom had gallstones, and two kinds of patient controls (with gallstones and without gallstones) were obtained from a hospital in Chile. Each GBC case was assigned an age- and sex-matched control. Chilean medical students questioned the patients using a questionnaire that surveyed more than 300 items regarding lifestyle. Although Serra et al. (30) reported a preliminary analysis of this data (in Spanish) on all 90 cases, Endoh et al. (31) selected 74 female cases and analysed them using a conditional multiple logistic model.

Of the various positive findings, only two interesting results will be reported here: patients with a history of constipation showed a significantly high odds ratio of 2.16 (95% confidence limits, 1.01–4.38), and patients who consumed red chilli once or more times a day had an elevated risk of 2.16 (1.27–3.66) versus patients who consumed red chilli less than once a day and 4.66 (1.63–13.40) versus non-consumers. More details of the above results are described elsewhere (31). This finding prompted us to conduct studies on the effects of capsaicin and other unknown ingredients in chilli pepper to determine whether they have mutagenic or carcinogenic activities (32, 33).

3. p53 analysis—We first reported p53 gene alterations in a Japanese study in which 5 (31.3%) of 16 GBC cases exhibited an alteration (34). All 5 cases were single point mutations: 4 of the mutations resulted in an amino acid substitution and the other was a single base-pair deletion. Yokoyama et al. (35) examined 22 GBC cases in Japan and confirmed our findings. Using paraffin-block specimens collected in Santiago, Chile, they discovered an interesting difference in the types of alterations that were observed. In the Chilean samples, all 12 mutations were transitions, with 4 arising at CpG sites. In the Japanese specimens, 9 transitions, and 4 transversions were observed among 13 mutations. In addition, none of the transitions in the Japanese specimens were found at the CpG sites. These findings suggest regional differences in p53 mutagenesis.

**Further studies in Japan and Chile**

Priority should be given to the testing of the multifactorial causation hypothesis using data obtained after the government’s ban on CNP use. At present, a rapid reduction in mortality rates, especially for females with BTC and in data analysed according to birth cohorts is evident in the vital statistics data for Japan. With regard to the geographical clustering of BTC in Japan, the SMRs for BTC in males and females in Niigata dropped to the 5th and 7th highest positions, respectively, among 47 prefectures. We are currently examining changes in the clustering pattern within the prefecture of Niigata.

Secondly, a more detailed understanding of the natural history of GBC from the viewpoints of genetic and environmental interactions is needed. Genetic susceptibilities, such as HLA-DR4 or CYP1A1 gene polymorphisms, appear to be involved in the occurrence of GBC. Since the dendrogram of HLA-DR antigens shows that the Japanese and American Indians belong to the same cluster, the analysis of genetic susceptibility is of great importance. However, differences in the risk factors for GBC and in p53 gene alterations were observed between Japan and Chile. An ethnic epidemiological approach may help to increase understanding of GBC.

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