Wim Van Isterdael

From: Eliane.Kellen@med.kuleuven.be
Sent: dinsdag 2 juni 2009 13:26
To: Biomedische bibliotheek
Cc: Eliane Kellen
Subject: e-Delivery request --- ID: 53385

Electronic delivery !! **ID: 53385**

NAAM:	Kellen, Eliane
DATUM:	02/06/2009
BUDGETVERANTWOORDELIJKE:	Heyrman, Maatsch. Gez. / ACHG - Acad. Centr. voor Huisartsgeneeskun
TITEL TIJDSCHRIFT:	Acta clinica Belgica
JAAR:	~~2009~~ 2008
VOLUME:	63
NR:	5
BLZ:	313-20
AUTEUR/TITEL ARTIKEL:	The investigation of a geographical cluster of bladder cancer.

Indien het artikel niet aanwezig is, plaats een interbibliothecaire aanvraag via:

Binnenland:	NEE
Buitenland-Köln:	NEE
Buitenland:	NEE

Original Article

THE INVESTIGATION OF A GEOGRAPHICAL CLUSTER OF BLADDER CANCER

E. Kellen[1], M.P. Zeegers[1,2], L. Bruckers[3], F. Buntinx[1,4]

Key words: : geographical cluster, bladder cancer, case control study.

ABSTRACT

Previous analyses of the Limburg Cancer registry (LIKAR) indicated the existence of a geographical cluster of bladder cancer incidence, particularly transitional cell carcinomas, amongst males in the surrounding area of the Belgian cities Hasselt and Alken. In subsequent ecologic analyses no risk factors were identified which could explain the existence of this cluster. Therefore, an epidemiological case-control study has been performed in the province of Limburg to explore the possible determinants of the cluster. The following cluster determinants were investigated: socio-demographic characteristics, life style factors, occupational and environmental exposures, and genetic predisposition. A weight was assigned to all the study subjects. Secondly, we used unconditional logistic regression to calculate crude odd ratios (ORs) and 95% confidence intervals (CIs) for each source of exposure and bladder cancer. Thirdly, we used a multivariate logistic regression analysis which included all the parameters found to be significant at the 0.1 significance level in the univariate analysis. Although some of the specific odds ratios decreased compared with the crude results, the overall excess did not change specifically. In conclusion, we were not able to identify a clear-cut explanation for the existence of the geographical cluster. We therefore recommend an increased surveillance of bladder cancer incidence for 3 to 5 years. If the "excess" of cancer cases has occurred due to random variation, the rate will return to the "expected" range in the next years. If the rate remains elevated, further studies may need to be performed using investigative tools that are not available currently.

INTRODUCTION

The province of Limburg is situated in the north-eastern section of Belgium and covers 2422 square km. The population is estimated at 798,583 inhabitants (2002) and is relatively young: 25% is younger than 20 years, only 17% is older than 60 years. In north and middle Limburg the major economical activities are related to the petrochemical, electronic and automobile industry. In southern Limburg fruit farming remains one of the major economical activities. LIKAR (the Limburg Cancer registry) was founded in 1993. Its major objective is to follow the incidence and trends of histologically or cytologically confirmed cancers of all inhabitants of Limburg and to continuously analyse the collected data. These data are provided by all the pathological, cytological and haematological laboratories

[1] Academisch Centrum Huisartsgeneeskunde, Katholieke Universiteit Leuven – Limburgs Kankerregister, België;
[2] Unit of Genetic Epidemiology, Department of Public Health and Epidemiology, University of Birmingham, United Kingdom;
[3] Centrum voor statistiek, Universiteit Hasselt, België;
[4] Huisartsgeneeskunde en Research Institute Caphri, Universiteit Maastricht, Nederland

Address for Correspondence:
Eliane Kellen
Academisch Centrum voor Huisartsgeneeskunde
Kapucijnenvoer 33, Blok J
3000 Leuven
Belgium
Fax: +32 (0)16 33 74 80
E-mail: Eliane.Kellen@med.kuleuven.be

located in the province and some outside the province which more regularly examine samples from Limburg inhabitants. Sex-stratified and age-standardised incidence rates have been calculated for each municipality and for most cancers in order to identify possible clusters of increased cancer incidence. Analyses within the LIKAR- register learned that bladder cancer is the fifth most common cancer among males (1996-2000) in this province. The male/female ratio is approximately five to one. Very few cases occur under the age of 40 years with two thirds of the cases occurring over the age of 65 years (1). Analyses of the data indicated a geographical cluster of bladder cancer incidence amongst males in the surroundings of Hasselt and Alken (2). Using a conditional autoregressive model, smoothed standardised incidence ratios (SIR) above 1.5 were identified in all municipalities of the cluster with a SIR of 2 in Alken, the municipality with the highest SIR ($p< 0.01$) .

Also in females similar or higher age-standardised SIRs were found in these municipalities. These disappeared, however, after smoothing. This was probably due to a lower power resulting from a lower number of bladder cancer cases in women. When focusing on transitional cell carcinomas (TCC), (82% of all bladder cancers), the results were even stronger. Five municipalities with a SIR above 2.0 within this cluster could be identified (2). Following the discovery of the bladder cancer cluster a further ecologic analysis of the data was performed. The standardized incidence ratio of male bladder cancer of each municipality was related to an index of the degree of urbanization by linear regression and no relation was found. However, it was found that the incidence rate was related to a municipality-specific index of the socio-economic status (SES). A higher standardized incidence rate was found in municipalities with a higher SES. This explained 11% of the variance of the incidence rates. No relationship was found with the proportion of migrants per municipality or the proportion of 'ever-smokers' in the municipalities (1).

Hence, an epidemiological study with the individual as unit of analysis was warranted.

A case-control study has been performed in the province of Limburg to explore the possible determinants of the cluster.

MATERIAL AND METHODS

Study population

The methods have been described in detail previously (3). We conducted a population based case-control study among 172 cases and 228 controls randomly distributed, in the Belgium province of Limburg among those 29 cases and 44 controls lived within the region of the cluster. All participants were Caucasian males. All cases were diagnosed with histologically confirmed transitional cell carcinoma of the bladder between 1999 and 2004. Cases were selected from the Limburg Cancer Registry (LIKAR) and approached through urologists and general practitioners. Due to the strict privacy law in Belgium, the population register is not directly accessible to researchers. A request was made to the "Kruispuntbank" of the social security for simple random sampling, stratified by municipality and socio-economic status, among all citizens above 50 year of age of the province. An invitation letter was sent to the selected subjects through the "Kruispuntbank". The study was approved by the ethical review board of the Medical School of the Catholic University of Leuven. Informed consent was obtained from all study subjects.

Interview and data collection

Three trained interviewers visited cases and controls at home. Information by structured interview was obtained on medical history, lifetime smoking history, family history of bladder cancer, 20-year residential history, a standardised food frequency questionnaire and a lifetime occupational history. The life-time occupational history listed all jobs (including official jobs and jobs done outside normal working hours) lasting more than six months and consisted of the job title, the industry or type of business, employment dates and duration, company name and location, tasks and materials used. Occupational exposure to PAHs, aromatic amines and diesel were blindly coded by two experienced occupational hygienists. Three different exposure categories were defined: no exposure, possible exposure, and nearly certain exposure.

A standardised food frequency questionnaire, derived from the IMMIDIET study was used to register nutritional characteristics (4). The IMMIDIET questionnaire was developed in the province of Limburg. The food frequency questionnaire contains 788 food items and was based on three existing food tables (the NEVO table of the Netherlands, the Nubel table of Flanders and the IPL table of Francophone Belgians) (5-7). For each individual food item, participants reported their frequency and the quantity of intake. The questionnaire was sent by mail to the participants of the study. During the home visit, the interviewer reviewed the answers, possibly correcting or completing some questions. After the questionnaire was electronically scanned, a com-

puterised program provided linkage to food composition tables.

All addresses, noted in the 20 year residential history, were geocoded. Based on these indicators for environmental exposures including exposures to arsenic, pesticides and landfills were calculated.

Biological samples

Additionally to the personal interview, biological samples were collected. Preference was given to blood samples. However, if this was refused by the participant, buccal swabs were collected. This was the case for less than <5% of the participants. Genomic DNA was extracted from peripheral blood lymphocytes or buccal swabs using standard methods at the Genome Centre Maastricht. DNA was resuspended in Tris-EDTA (TE) buffer and stored at –20°C for future use. Genotyping was done for *GSTM1, GSTT1, NAT2* and *SULT1A1 R213H*. The methods have been described previously (8).

Blood cadmium, arsenic, lead, zinc and selenium concentrations of cases and controls were analyzed in random order, with the case status unknown to the laboratory staff (Algemeen Medisch Laboratorium, Antwerp) were determined by plasma mass spectrometry (accreditation certificate of the Belgian government).

Statistical analysis

Our analytical plan involved three steps. Firstly, since the geographic distribution of the cases and the controls in the study did not reflect the one of the Limburg population nor of the bladder cancer cases noted in the cancer registry, a weight was assigned to all the study subjects based on the population figures (2001) and the number of bladder cancer cases per municipality (1996-1998). The municipalities were divided in two groups: in and outside the cluster. Per group and age-group (<49 <59, <69, <79, 80+), the rate of the number of study cases to the number of bladder cancer cases in the real population was calculated. The inverse of the calculated rate equalled the weight assigned to the cases of a specific stratum. This calculation was repeated for the controls. Secondly, we used unconditional logistic regression to calculate crude odd ratios (ORs) and 95% confidence intervals (CIs) for each source of exposure and bladder cancer. This correction was performed first through analyses including two parameters; i.e. the cluster indicator and one additional explanatory variable (we refer to these models as univariate models). Thirdly, we used a multivariate logistic regression analysis which, besides the cluster status, included all the parameters found to be significant at the 0.1 significance level in the univariate analysis. Based on the distribution of the data and known risk factors of bladder cancer we adjusted for age and smoking. Adjustment for smoking was done by creating three new variables: current cigarette smoking status, years of cigarette smoking and number of cigarettes smoked per day.

All analyses were conducted in SAS version 9.1. The weighed analysis was performed by means of PROC SURVEYLOGISTIC.

RESULTS

The OR for the existence of the cluster was 2.78 (95%CI 1.59-4.85). We refer to this OR as the crude OR, since no correction for possible differences in the composition of the persons living in and out the cluster was done.

After the second step of the statistical analysis did the overall excess not change specifically, although for some parameters the inclusion in the weighted logistic regression model led to a reduction of the OR for the cluster,

Next, all parameters significant in the univariate models, were included in a multivariate model. The complex model, including all variables significant at the 0.1 level in the univariate models, was simplified via a backward selection procedure, until all parameters were significant at the 0.05 level. The Wald test was used for this purpose.

Age and smoking were included regardless the significance level. The cluster effect was not statistically in this model (p=0.17). However, the magnitude of the OR for the cluster effect did remain at the same level of the crude OR.

Table 2 presents the removing of the variable from the complex model. The value of the Wald test, the degrees of freedom and the p value at each step are given. Finally nine variables were left in the model (the cluster (in versus out), education (categorized), age (categorized), number of cigarettes smoked a day, cakes, arsenic, selenium, cadmium and landfills).

DISCUSSION

Our aim was to use case-control data to investigate the reasons of a geographical cluster of male bladder cancer in the cluster region around Hasselt and Alken. To investigate the relation between bladder cancer in-

cidence and residential location, we took into account individual risk factors and used an individual level of analysis.

Our study had several methodological strengths. We were able to control for many covariates; like most cancers, bladder cancers are likely to be caused by a combination of factors related to genetics and environment (including behaviour and lifestyle). We investigated risk factors, which may be heterogeneously distributed; including occupational and environmental exposure, smoking habits and socio-economic status. For cancers with a long latency time like bladder cancer, migration rates will make any spatial patterns associated with potential exposures less obvious. However, the 20 –year residential history information allowed us to take latency into account when considering environmental exposures. Latency was also taken into account by noting down a lifetime smoking and occupational history. Furthermore, the calculation of the environmental exposures was based on geocoded addresses and is therefore not prone to recall bias as the exact address was noted. Nevertheless, our results have a number of potential limitations. As in any case-control study, the loss of patients who could not be interviewed due to high age or to the seriousness of their disease might introduce selection bias. Unfortunately, the privacy law also did not permit us to receive any socio-demographics characteristics of the selected subjects. Therefore, we were only able to compare the sex and age distribution of the controls with those of the general population. A major limitation of case-control studies is the possibility of differential recall of past events (recall bias) among cases and controls. Cases may overreport or underreport their actual intake if they are aware of the exposure-disease relationship of interest. A residential history does not account for daily movement of individuals. Subjects may be environmentally exposed through home-work traffic. Hasselt is capital of the province and provides work for many inhabitants of surrounding cities. Pollution through traffic is therefore expected to be high in Hasselt. Finally, establishing significant and valid evidence that a specific genetic factor leads to an increased chance that a specific environmental exposure will result in cancer may require a larger study over a long period of time.

Although in the final model, the cluster is not statistically significant; we were not able to find an identifiable cause for the observed bladder cancer cluster.

Indeed, much debate in the literature remains whether or not a cluster should be investigated (9). It has been argued that virtually all diseases show spatial clustering. Therefore, there is little public health purpose to investigate individual disease clusters and little reason to study overall patterns of disease in space-time (10). However, the sex-stratified and age-standardised incidence rates of all other cancers in the LIKAR registry did not detect a geographical cluster. Case-control studies of clusters have often failed to identify new risk factors. Case-control studies carried out to an observed excess of cases are subject to several limitations: the role played by random fluctuations in the occurrence of the cluster; the ability to identify a potential risk factor; and the ability to evidence a real link between environment and the disease (11).

But, until clinical or molecular tests are developed that determine the cause of cancer in an individual, researchers must rely on epidemiologic studies that can identify factors associated with risk in groups of people (12). Furthermore, conditions for fruitful cluster investigations have been proposed (13). Our bladder cancer cluster met several of these conditions. The pathophysiologic mechanism of the agents responsible for bladder cancer is well understood. Tobacco is considered to be the main risk factor. Approximately half of male urinary tract cancer and one-third of female urinary tract cancer might be attributable to cigarette smoking (14). Occupational exposure, particularly to aromatic amines and polycyclic aromatic hydrocarbons (PAHs), may play an important role in perhaps 10% of bladder cancers (15). More than 60 carcinogens have been described in cigarette smoke. Among these are PAHs (such as benzo(a)pyrene) and aromatic amines (such as 4-aminobiphenyl) which have been formally evaluated by the International Agency for Research on Cancer, and in-vivo studies in either laboratory animals and humans have provided sufficient evidence of carcinogenicity (16). Occupational exposure to PAHs and aromatic amines is rare in the normal population and there may be heterogeneity of exposure within the neighbourhood. Occupational exposure and smoking habits can be assessed through a questionnaire. Our bladder cancer cluster was a to date uninvestigated endemic space cluster instead of a space-time cluster.

For most observed cancer clusters in the literature, an identifiable explanation can not be found (17). Of course, the influence of factors unknown to investigators cannot be assessed directly. Starting in the early 1960s, the Centre for Disease Control investigated 108 cancer clusters; no clear cause was found for any cluster (18). However, for several previously unexplained cancer clusters it has been demonstrated that they can be at-

tributed to exposures of chemical mixtures rather than one individual chemical (19). But, even when, a preventable cause of a cancer was not identified, cancer prevention and control experts can promote education and screening in a community concerned about a cancer cluster (17). Finally, it is important to note that cancer rates may be elevated in some geographical areas purely due to chance.

In conclusion, we were not able to identify a clear cut explanation for the existence of a geographical cluster of bladder cancer. Therefore, we recommended an increased surveillance for three to five years. Eight years of incidence rates of cancer in Limburg have become available very recently. The geographical was still significant statistically.

As the rate has not returned to the "expected "range , the "excess" of cancer cases has not occurred due to a random variation, Since the rate remains elevated, further studies may need to be performed using investigative tools that are not available currently.

ACKNOWLEDGEMENTS

This project was funded by the Flemish Government., the Belgian province of Limburg and the 'Limburg Kankerfonds'. We thank N. Knoors and R. Reulen for their valuable contribution to the data collection.

We are grateful for the kind cooperation of the following pathologists: L. Op De Beeck, J. Vanden Brande, M. Vanbockrijk, J. Vandepitte, L. Capelle, L. Wouters, J. Van Robaeys, R. Stessens, P. Van Eyken , A. Cornelis and L. Peperstraete. We thank the following urologists and general practitioners for case identification: W. Vervecken, K. Dilen, H.Goethuys , D. Herremans , P. Martens, P. Meylaerts, S.Schapmans, J.Stragier , J.Vanderkerken, J.Van Nueten, K. Van Renterghem, K. Verveken, P. Willemen, M. Jacobs, K. Leyssens, M. Viane, A. Van Erum, J. Martens, F. Suckers, P.Roussel, H. Medaer, S. Hendrickx, L.Deferm, P. Sauwens, B. Bemelmans, P. Claes, M. Daniels, M. Loos, J. Vanhoof, J. Mertens, D. Vantroyen, F. Luyckx, B. Moors, M. Buttiens, H. Goossens, D. Vandeweerd, J. Van Holderbeke, F. Blauwens, C. Gilio, S. Rigo, E. Vanderputte, R. Orye, P. Dexters, R. Vijgen. We acknowledge the valuable suggestions of R.Janssen, B. Adriaens, E. Cloes, A. Forrier, H. Geys, T. Hermans, D. Lousbergh, F. Luyckx, J. Mebis, G. Thijs, J. Van Meerbeek, S. Callens, G. Moens, W. Oosterlinck and J. Van den Broucke as members of the steering group and the scientific advisory board. We thank M. Lismont and M. Devis for technical assistance.

ABSTRACT

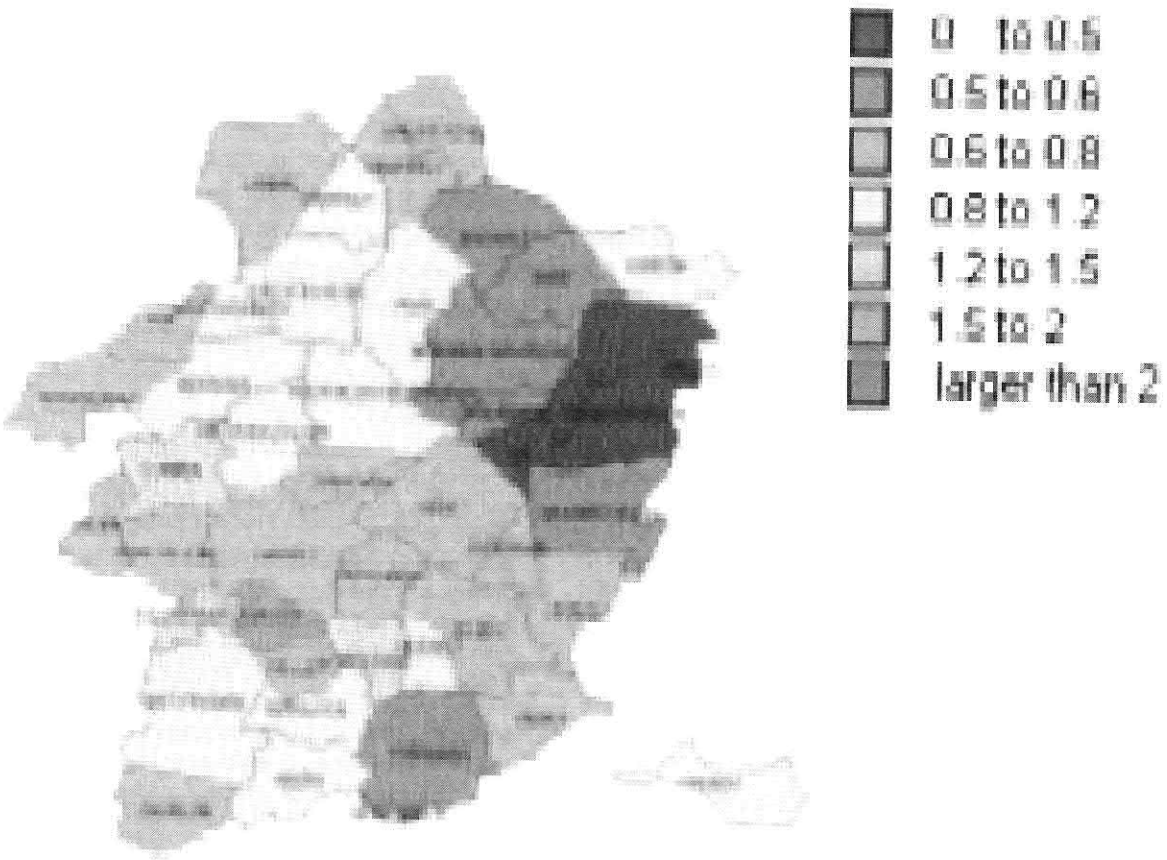
Analyses op basis van het Limburgs Kanker register LIKAR toonde een geografische cluster van blaaskanker bij mannen aan. De hoogste waarde werd gevonden in de regio Hasselt-Alken. Er wordt aangenomen dat de absolute cijfers bij vrouwen te klein zijn om tot dezelfde conclusie te komen. De resultaten bleken nog duidelijker te zijn wanneer enkel gekeken werd naar het histologisch type transitoneel cell carcinoma. Deze verhoogde waarden konden niet verklaard worden door allerlei karakteristieken van de betrokken gemeenten. Daardoor werd besloten tot een epidemiologisch onderzoek met het individu als eenheid van analyse. Er werd specifiek gekeken naar socio-demografische karakteristieken, leefstijlfactoren, beroepsblootstellingen en genetische polymorphismen. Een gewicht werd toegekend aan alle personen. Door middel van logistische regressie werden odds ratio's (OR) en 95% betrouwbaarheidsintervallen berekend voor elke blootstelling en blaaskanker. Vervolgens werd een mulitvariate analyse uitgevoerd voor alle factoren, die significant waren in de univariate analyse.

Aangezien we niet in staat zijn de cluster eenduidig te verklaren, raadden wij aan om de incidentie van blaaskanker nauwgezet te volgen gedurende drie tot vijf jaar. Indien de cluster het gevolg was van een toevallige variatie, zal de incidentie de komende tijd dalen. Indien dit niet gebeurt, is verder onderzoek nodig.

REFERENCES

1. Lousbergh D, Broeders G, Cloes E, et al. Incidence of cancer in the Begian province of Limburg (1996-2001). 2003. Limburgs Kanker Register.
2 Buntinx F, Geys H, Lousbergh D, et al. Geographical differences in cancer incidence in the Belgian province of Limburg. *Eur. J.Cancer* 2003;39(14):2058-72.
3. Kellen E, Zeegers MP, Lousbergh D, Paulussen A, Buntinx F. A Belgian case control study on bladder cancer: rationale and desing. *Archives of Public Health* 2005;63(1):17-34.
4. Iacoviello L, Arnout J, Buntinx F, et al. Dietary habit profile in European communities with different risk of myocardial infarction: the impact of migration as a model of gene-environment interaction. The IMMIDIET Study. *Nutr.Metab Cardiovasc.*Dis 2001;11(4 Suppl):122-6.
5. NEVO table 2001: Dutch composition table. 2001. The Hague: The Netherlands Nutrion Centre.
6. VZW Nubel. Belgian foodtable; third edition. 1999. Brussels: VZW Nubel.
7. IPL : Table de composition des aliments. 2000. Brussels: Institut Paul Lambin.
8. Kellen E, Zeegers MP, Paulussen A, Van Dongen M, Buntinx F. Fruit consumption reduces the effect of smoking on bladder cancer risk; the Belgian case control study on bladder cancer. *Interna-*

tional Journal of Cancer 2006 May 15;118(10):2572-8.
9. Disease clustering: hide or seek? *Lancet* 1990;336(8717):717-8.
10. Rothman KJ. A sobering start for the cluster busters' conference. Am J Epidemiol 1990;132(1 Suppl):S6-S13.
11. Bellec S, Hemon D, Clavel J. Answering cluster investigation requests: the value of simple simulations and statistical tools. *Eur J Epidemiol* 2005;20(8):663-71.
12. Thun MJ, Sinks T. Understanding cancer clusters. *CA Cancer J Clin* 2004;54(5):273-80.
13. Neutra RR. Counterpoint from a cluster buster. Am J Epidemiol 1990;132(1):1-8.
14. Zeegers MP, Tan FE, Dorant E, van den Brandt PA. The impact of characteristics of cigarette smoking on urinary tract cancer risk: a meta-analysis of epidemiologic studies. *Cancer* 2000;89(3): 630-9.
15. Kogevinas M, Trichopoulos D. Urinary Bladder Cancer. In: Adami H-0., Hunter D., Trichopoulos D., editors. Textbook of Cancer Epidemiology. New York: Oxford University Press; 2002. p. 446-66.
16. Hecht SS. Tobacco carcinogens, their biomarkers and tobacco-induced cancer. *Nat Rev Cancer* 2003;3(10):733-44.
17. Aldrich T, Sinks T. Things to know and do about cancer clusters. *Cancer Invest* 2002;20(5-6):810-6.
18. Caldwell GG. Twenty-two years of cancer cluster investigations at the Centers for Disease Control. *Am J Epidemiol* 1990;132(1 Suppl):S43-S7.
19. Zeliger HI. Unexplained cancer clusters: common threads. *Arch Environ Health* 2004;59(4):172-6.



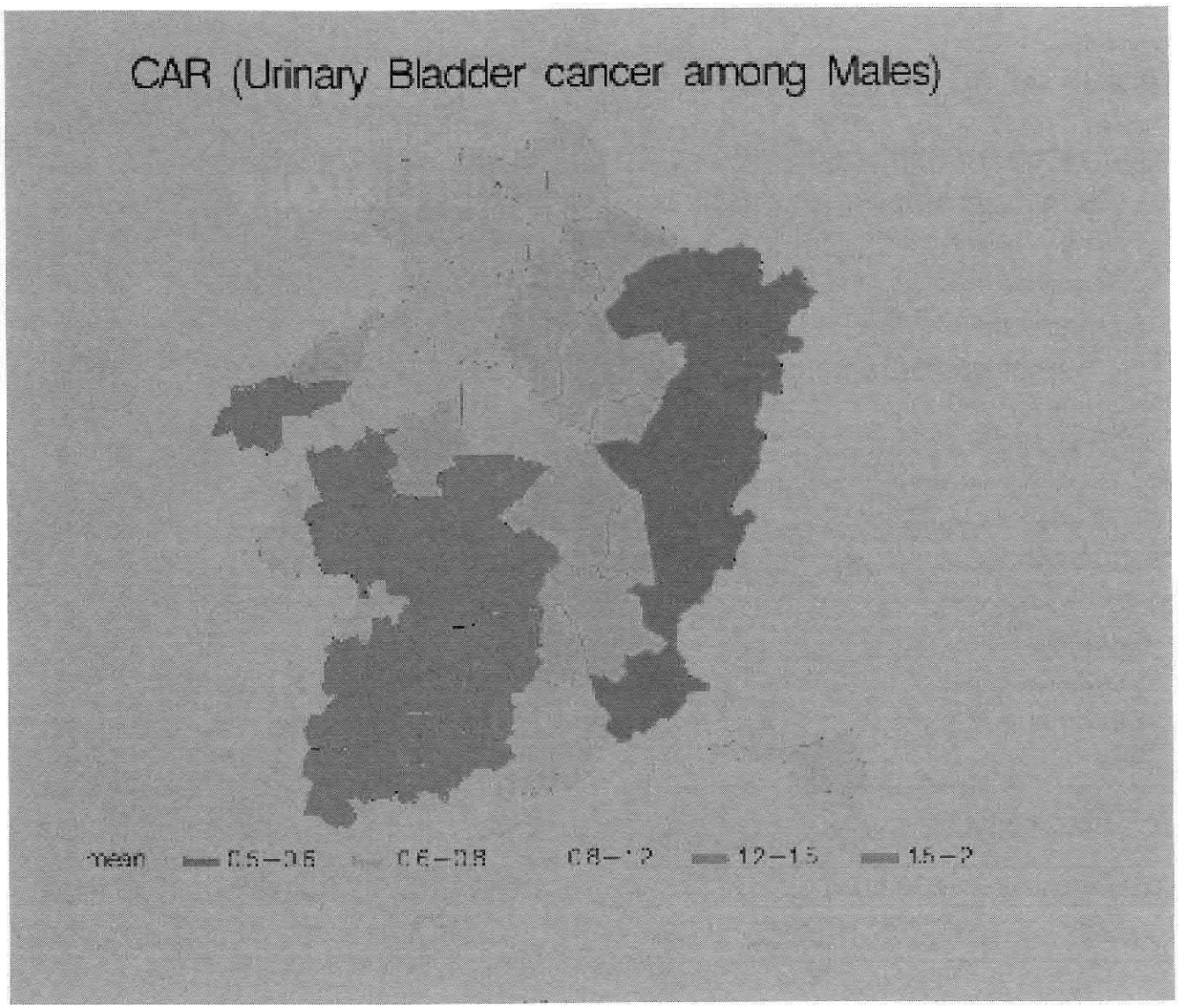

CAR (Urinary Bladder cancer among Males)
mean
0.5–0.6
0.6–0.8
0.8–1.2
1.2–1.5
1.5–2

Table 1: Odds ratios and 95% confidence intervals of parameters that were significant in the univariate analysis

		OR	95%CI
Cluster	Inside vs Outside	2.47	0.68-8.92
Education level	Elementary vs occupational training	0.63	0.13-3.25
Education level	High school vs occupational training	4.20	0.99-17.72
Education level	Superior vs occupational training	2.58	0.50-13.16
Education level	University vs occupational training	0.10	0.00-3.32
Age group	60-63 vs ≤60	16.58	2.87-95.66
Age group	65-69 vs ≤60	34.00	6.28-183.94
Age group	70-74 vs ≤60	97.94	13.93-668.48
Age group	≥75 vs ≤60	146.713	21.40->99.99
Ever smoking of cigarettes?	Yes vs no	1.02	0.97-1.06
Number of cigarettes smoked a day		0.51	0.06-3.96
Daily consumption of vegetables (g/day)		1.04	0.99-1.09
Daily consumption of fruits (g/day)		1.00	0.99-1.00
Daily consumption of cereals (g/day)		1.00	0.99-1.00
Daily consumption of fish (g/day)		1.00	0.99-1.00
Daily consumption of cakes (g/day)		1.00	0.99-1.00
Daily consumption of alcohol (g/day)		0.99	0.98-1.00
Daily consumption of miscellaneous (g/day)		0.99	0.98-1.00
Blood level of peroxide (µmol/l)		1.00	0.99-1.02
Blood level of arsenic (µg/l)		1.24	1.08-1.42
Blood level of cadmium (µg/l)		8.23	2.15-31.49
Blood level of selenium (µg/l)		0.98	0.96-0.99
Landfills		3.55	1.04-12.13

Table 2: Results of model fitting

Parameter	Wald Chi-Square	Degrees of freedom	p-value
Vegetables	.0472	1	.8279
Miscellaneous	.1122	1	.7376
Fish	.0706	1	.7905
Ever smoked (yes, no)	.5413	1	.4619
Fruits	.2376	1	.6259
Perioxide2	1.0536	1	.3047
Total years of smoking	.4775	1	.4895
Alcoholic beverages	2.0248	1	.1547
Cereals	1.6598	1	.1976