CONCENTRATIONS OF ORGANOHALOGEN COMPOUNDS AND TITTERS OF ANTIBODIES TO EPSTEIN-BARR VIRUS ANTIGENS AND THE RISK FOR NON-HODGKIN LYMPHOMA

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Introduction

The incidence of non-Hodgkin lymphoma (NHL) increased substantially during the second half of the 20th century in Sweden and in other Western countries. However, during the last decade the increasing incidence has levelled off in many countries.[3] This change of the incidence may be explained by decreasing exposure to certain risk factors in the population.

The established risk factors for development of NHL include different immunosuppressive states, e.g., HIV, autoimmune diseases as Sjögren´s syndrome and SLE, immunodepressants used after organ transplantation and some inherited conditions.[1] However, these factors can not explain the change of the incidence. Instead it is of interest that exposure to certain persistent organic pollutants, pesticides and organic solvents has been implicated to be of etiologic significance.[2,4,5] An interaction between Epstein-Barr virus (EBV) and pesticides has been shown in some studies.[4,8,9,11]

The purpose of this study was to determine concentrations of organohalogen compounds in patients with NHL and population based controls. The concentrations of these substances were correlated to titres of antibodies to EBV. The responsible ethical committees approved the study.

Materials and Methods

The study covered four (Umeå, Örebro, Linköping, Lund) health service regions in Sweden and data were collected during a period from December 1, 1999 to April 30, 2002. All consecutive patients with newly diagnosed NHL aged 18-74, identified through physicians treating lymphoma and through pathologists diagnosing the disease, were approached. All of the diagnostic pathological specimens were scrutinised to confirm the diagnosis.

From registries covering the whole Swedish population randomly chosen controls living in the same health service regions as the cases were recruited during several occasions within the study period. The controls were frequency matched to the cases and chosen within 10 years age groups to approximately mirror the age and sex distribution of the included cases.

This study was part of a larger case-control investigation on risk factors for NHL including 995 cases of which 910 (91 %) participated. Of the 1108 enrolled controls 1016 (92 %) answered the questionnaire, for more details see Hardell et al (2005).[6] Blood samples for this part of the study was obtained from a subset of cases at the participating university hospitals and control subjects.

In total 203 cases and 254 controls were included. All samples got a unique id-number that did not reveal if it was a case or a control. From this pool of specimens 100 cases were drawn at random and 100 controls fulfilling the matching criteria (sex, 10-years age group). Thus this study encompassed in total 200 subjects.

Chemical analysis

2 ml of each sample was extracted by solid phase extraction, using 200 mg ENV+ columns. In addition, one laboratory blank sample and one reference sample of each set of 6 samples were analysed. The lipid content of each sample was determined enzymaticaly from a sub-sample. The samples were fortified with ^{13}C-labelled internal polychlorinated biphenyl (PCB) and polybrominated diphenylether (PBDE) standards.

Congener specific analyses and quantification of the analytes was performed on an Autospec Ultima HRGC-HRMS, running in SIR mode, using EI ionization at 35 eV. The two most abundant ions of the chlorine cluster of the molecular ion for each compound were measured, as well, as the ions for the ^{13}C labelled internal and recovery standards. A quantification standard mixture including all compounds in addition to the IS and RS was used to calculate relative response factors (RRF). These RRFs were used to calculate the compound levels in the samples. All data was analysed with Mass-Lynx software and all calculations were made with Quan-Lynx.

EBV antigens
Indirect immunofluorescence was used for IgG antibodies to the virus capsid antigen (VCA) and the combined restricted and diffuse components of early antigens (EA R+D). The VCA IgG antibodies were end-point titrated in four-fold dilutions from 1/20, while EA IgG was analysed in one dilution, allowing for detection of antibodies in dilution $\geq$ 1/20 (titre $\geq$ 1/20). Positive samples were end-titrated.

Statistical Methods
Unconditional logistic regression analysis was performed using the Stata program (Stata/SE 8.2 for Windows; StataCorp, College Station, TX) for calculation of odds ratio (OR) and 95% confidence interval (CI). In the analyses adjustment was made for age as a continuous variable, sex and Body Mass Index (BMI) at the time of sampling.

The median concentrations of antigens and organohalogenated compounds in the controls were used as cut-off values in the calculations of ORs and CIs since no biological relevant cut-off exists. The Stata program was also used for descriptive statistics and Wilcoxon rank sum tests for calculation of *p*-values.

Results
In Table 1 results are presented for the concentrations of organohalogen compounds in cases and controls. Significantly higher concentrations ($p<0.05$) were found in the cases for sum of PCBs, hexachlorobenzene (HCB) and sum of chlordanes. Regarding p,p'-dichlorodiphenyldichloroethyelene (p,p'-DDE) and PBDE no significant differences were found between cases and controls.

Table 1. Concentrations of organohalogen compounds (ng/g lipid) in cases and controls. Wilcoxon p-value is given.

	Number	Mean	Median	Min	Max	p
sum of PCBs						
— cases	97	860	762	102	4222	0.02
— controls	98	672	646	122	2005	
HCB						
— cases	99	31	26	0.42	140	0.01
— controls	99	27	24	0.34	215	
p,p'-DDE						
— cases	99	486	307	5.4	2786	0.11
— controls	99	343	271	17	1414	
sum of chlordanes						
— cases	99	31	26	2.7	110	0.03
— controls	99	25	19	3.4	101	
PBDE						
— cases	99	2.8	1.5	0.20	79	0.30
— controls	99	2.7	1.8	0.005	25	

Results of analysis of interaction between titre of EA IgG and organohalogens are presented in Table 2. For all studied compounds except for PBDE an interaction was found with highest OR in the high concentration group and titre to EA IgG > 40.

Table 2. Odds ratio (OR) and 95 % confidence interval (CI) for different organohalogen compounds and NHL in relation to titre to Epstein-Barr virus early antigen (EA) IgG. As cut-off the median concentration of the chemicals and titre to EA in the controls was used. Numbers (expressed in ng/g lipid) are shown for cases and controls. Adjustment was made for age, sex and BMI.

Exposure	Cases / Controls	OR, CI
EA ≤ 40, sum of PCBs ≤ median	14 / 25	(1.00)
EA > 40, sum of PCBs ≤ median	24 / 24	– 2.49 (0.97 – 6.37)
EA ≤ 40, sum of PCBs > median	20 / 29	2.11 (0.73 – 6.07)
EA > 40, sum of PCBs > median	39 / 20	5.25 (1.91 – 14.4)

EA ≤ 40, HCB ≤ median	9 / 24	(1.00)
EA > 40, HCB ≤ median	34 / 26	– 3.85 (1.47 – 10.1)
EA ≤ 40, HCB > median	25 / 30	2.51 (0.94 – 6.70)
EA > 40, HCB > median	31 / 19	5.27 (1.87 – 14.9)
EA ≤ 40, p,p'-DDE ≤ median	20 / 28	(1.00)
EA D > 40, p,p'-DDE ≤ median	26 / 22	– 1.77 (0.76 – 4.12)
EA ≤ 40, p,p'-DDE > median	14 / 26	1.04 (0.40 – 2.71)
EA > 40, p,p'-DDE > median	39 / 23	3.26 (1.38 – 7.73)
EA ≤ 40, sum of chlordanes ≤ median	13 / 23	(1.00)
EA > 40, sum of chlordanes ≤ median	24 / 27	– 1.83 (0.70 – 4.75)
EA ≤ 40, sum of chlordanes > median	21 / 31	1.79 (0.63 – 5.10)
EA > 40, sum of chlordanes > median	41 / 18	6.78 (2.33 – 19.7)
EA ≤ 40, PBDE ≤ median	17 / 24	(1.00)
EA > 40, PBDE ≤ median	43 / 26	– 2.72 (1.19 – 6.23)
EA ≤ 40, PBDE > median	17 / 30	0.94 (0.39 – 2.32)
EA > 40, PBDE > median	22 / 19	1.81 (0.73 – 4.47)

Discussion

In this study we found significantly increased concentrations in NHL cases for the sum of PCBs, HCB and sum of chlordanes. An interaction between elevated EA IgG and these chemicals was found. Furthermore, in the high concentration group of p,p'-DDE and high titre to EA IgG OR = 3.26, 95 % CI =1.38–7.73 was obtained. When analysing the chemicals only without considering titre to EA IgG lower ORs were obtained. This suggests an interaction between these chemicals and EBV.

These results are similar to our previous study on this topic.[4] In that study, both blood and adipose tissue was used for the chemical analysis of organohalogen compounds. We now used plasma only, from both cases and controls.

EBV is a human herpes virus prevalent in B-lymphocytes. The virus is found worldwide and the majority of the adult population has antibodies to EBV antigens. The primary infection occurs usually in childhood and is usually sub clinical. A latent infection is established that is balanced by the host immune response. EBV has been associated with certain types of NHL such as Burkitt lymphoma and lymphomas in immunologically compromised or HIV-infects subjects.[10]

Immunosuppression is a risk factor for NHL and exposure to organochlorines has been reported to compromise the immune system in humans.[7] Immunosuppression may lead to loss of cell-mediated immune control of reactivated EBV and give clonal expansion of cells.

Cases were consecutively recruited from the participating clinics and pathology departments as soon as histopathological diagnosis was obtained. Thus it was possible to include the cases soon after diagnosis. Controls were selected at random from the Swedish population registry and selection bias was avoided.

All results were adjusted for age and sex. Furthermore adjustment was made for BMI since the studied compounds are lipid soluble and changes in body weight might influence the results. The blood samples were coded and during the chemical analysis it was not known if it was a case or a control. All results were lipid based. In summary this study showed an association between certain organochlorine compounds and NHL with an interaction with titre to EA IgG antibody to EBV.

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