15. Roed H, Aabo K, Vindelov L, Spang-Thomsen M, Christensen IBJ, Hansen HH. *In vitro* and *in vivo* evaluation of the indoloquinone EO-9 (NSC 382 459) against human small cell carcinoma of the lung. *Eur J Cancer Clin Oncol* 1989, 25, 1197–1201.
16. Alley MC, Scudiero DA, Monks A, *et al.* Feasibility of drug screening with panels of tumor cell lines using a microculture tetrazolium assay. *Cancer Res* 1988, 48, 589–601.
17. Rebai N, Malissen B. Structural and genetic analyses of HLA class I molecules using monoclonal xenoantibodies. *Tissue Antigens* 1983, 22, 107–117.
18. Pizao PE, Leyva A, Keepers Y, Peters GJ, Pinedo HM, Winograd B. *In vitro* sensitivity testing using multilayers in "V"-shaped microtiter plates to mimic *in vivo* conditions. *Proc Am Assoc Cancer Res* 1990, 31, 432.

Acknowledgements—This work was supported by the Dutch Cancer Society (Grant IKA 88-18). P.E.P. is a recipient of a grant from CNPq/Ministry for Science and Technology of Brazil. G.J.P. is the recipient of a senior research fellowship of the Royal Netherlands Academy of Sciences.

This work was performed within the framework of the EORTC–In Vitro Study and Screening Group.

Eur J Cancer, Vol. 27, No. 7, pp. 900–902, 1991.
Printed in Great Britain

0277–5379/91 $3.00 + 0.00

Selenium in Human Mammary Carcinogenesis: a Case-cohort Study

Kim Overvad, Dennis Y. Wang, Jørn Olsen, Diane S. Allen, Eivind B. Thorling, Richard D. Bulbrook and John L. Hayward

In a prospective study conducted on the island of Guernsey a cohort of 5162 ostensibly healthy women was enrolled between 1967 and 1976. Blood samples were drawn from each participant, who also completed a questionnaire, which provided information on established risk indicators in human mammary carcinogenesis. Plasma selenium levels were measured in 46 breast cancer cases diagnosed a mean of 11 (S.D. 4) years after entry into the study cohort and in an age-stratified sample of 138 women drawn from the study base. Plasma selenium level in the cases was 109 (28) μg/l and in the base sample 103 (22) μg/l (95% confidence interval for the overall difference, −2 to 14 μg/l). The adjusted relative risk of developing breast cancer in the different quartiles of the selenium distribution was 0.80, 0.79, 0.72 and 1.00, respectively. Thus, in the present study selenium was not a strong indicator of human breast cancer risk.
Eur J Cancer, Vol. 27, No. 7, pp. 900–902, 1991

INTRODUCTION

THE EPIDEMIOLOGICAL evidence pertaining to a relationship between selenium and human mammary carcinogenesis is confused. Studies on aggregated data revealed decreasing human breast cancer mortality rates at increasing levels of selenium exposure [1, 2]. Of three case-referent studies, two reported relatively low levels of blood selenium in the cancer patients [3, 4], while in the remaining study mean selenium levels among cases and referents were comparable, although the selenium distributions indicated a positive association between selenium levels and cancer risk [5].

Several historical prospective studies have examined the relation between measures of selenium exposure and cancer risk. One study on blood selenium levels indicated a positive association between selenium levels and breast cancer risk [6], while another study on nail selenium levels indicated no such association [7].

The present study was undertaken to examine the importance of selenium in human mammary carcinogenesis, considering breast cancer cases in which the diagnoses were made several years after collection of blood specimens to minimise the possibility of disease mediated changes in selenium levels.

MATERIALS AND METHODS

Between 1967 and 1976, a cohort of 5162 ostensibly healthy women, aged 35 years or more, was enrolled to investigate the importance of hormonal factors in human mammary carcinogenesis. The women, who lived on the island of Guernsey, had volunteered to take part in the epidemiological investigation. They were recruited by their doctors, from various women's organisations and by appeals in the local press and on the television. This cohort was derived from a population of approximately 13 000 eligible women.

At entry into the study cohort, non-fasting blood samples were drawn from each participant. Heparinised plasma was prepared and stored at −20°C. Each woman completed a questionnaire, which provided information on the following established risk indicators in human mammary carcinogenesis: date of birth, family history of breast cancer, age at menarche, number of children, age at first baby, breast feeding, age at menopause, height and weight. A record of diagnoses of breast

Correspondence to K. Overvad.
K. Overvad and J. Olsen are at the Institute of Social Medicine, University of Aarhus, Høegh-Guldbergs Gade 8, DK-8000 Aarhus C, Denmark; D.Y. Wang, D.S. Allen and R.D. Bulbrook are at the Department of Clinical Endocrinology, Imperial Cancer Research Fund, London, U.K.; E.B. Thorling is at the Danish Cancer Society, Department of Nutrition and Cancer, Aarhus Municipal Hospital, Aarhus C, Denmark; and J.L. Hayward is at the Breast Unit, Guy's Hospital, London, U.K.
Revised 4 Apr. 1991; accepted 9 Apr. 1991.

cancer developing in the cohort was established through the general practitioners or the pathologists of the island hospital or through a search of the death certificates. All diagnoses were confirmed histologically, and the histology was reviewed by an independent pathologist.

To evaluate the importance of selenium in human mammary carcinogenesis, plasma samples collected at the women's entry into the study cohort were analysed for selenium. Samples from women who developed breast cancer during the study period together with an age-stratified sample of the study base were analysed with a case-base ratio of 1:3.

Determination of plasma selenium levels was performed by particle-induced X-ray emission (PIXE) at the Institute of Physics, University of Aarhus [8]. 40 μl of plasma and 140 μl of an internal yttrium standard were thoroughly mixed, and 75 μl of the solution placed on a 8 μm porisity Nuclepore polycarbonate membrane mounted in an aluminium slide frame. The samples were dried in an oven at 50°C for 60 min. The mounted samples were placed in a slide carousel in the analysis chamber. Irradiation was performed with a 2.55 MeV proton beam with a beam diameter of 10 mm. The beam current was 30–50 nA and the total accumulated charge per spectrum was 150 μC. The X-rays were detected using a Kevex energy dispersive detector with an active area of 80 mm^2, and an energy resolution of about 180 eV. A 0.5 mm mylar absorber was placed in front of the detector window. The angle between the detector and the beam was 135°. A gold diffuser foil and a double pair of collimators were used to ensure a homogeneous beam spot. To reduce pulse pileup in the detector system, triggered beam pulsing was employed. The spectra were accumulated by a microcomputer and analysed using the HEX program [9]. An international standard, Seronorm 105 (Nycomed AS, Oslo, Norway), containing 90 μg selenium/l was used for calibration. The detection limit for selenium at the present set-up was about 30 μg/l. The coefficient of variation for the international standard was about 8%. All determinations were performed in duplicate and a similar coefficient of variation was calculated from the double determinations.

Table 1. Characteristics of the case and study base sample

	Cases	Referents
Age at entry	45.2 (10.0)	44.7 (10.4)
Age at diagnosis	56.1 (10.0)	
Marital status		
Single	21.7%	10.1%
Married	76.1%	82.6%
Other	2.2%	7.2%
Age at menarche	13.3 (1.6)	13.2 (1.4)
Parity		
0	37.0%	18.1%
1	13.0%	21.7%
2	21.7%	33.3%
3	28.3%	26.8%
Age at first baby	25.2 (4.4) (n = 29)	25.4 (4.5) (n = 113)
Family history of breast cancer	17.4%	12.3%
Height, cm	161 (7)	161 (6)
Weight, kg	64 (10)	63 (10)
Quetelet index	24.6 (3.6)	24.5 (3.8)

Mean (S.D.).

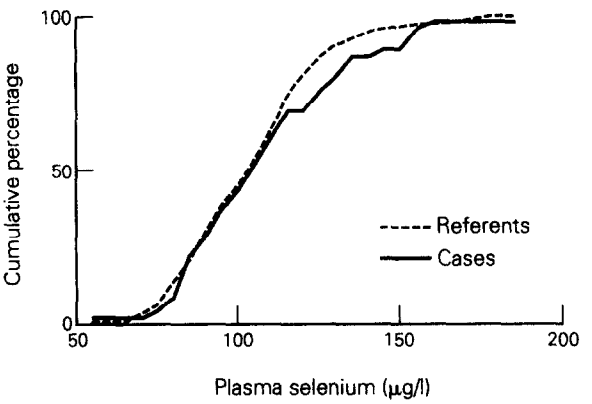

Fig. 1. Distributions of plasma selenium levels.

Statistical analyses of the results included description of the distribution among cases and among referents of the different variables included in the study. Initially possible associations between plasma selenium and potential confounders were evaluated separately. Finally, the results were evaluated using logistic regression.

RESULTS

Up to the end of 1985 a total of 88 women had developed breast cancer. No additional cancer cases were found at the examination of death certificates. Because most of the samples of the early cancers had been used up in endocrine studies, samples were only available from 24% of cases diagnosed before 1980 and from 78% of cases diagnosed from 1980 onwards. The present study was based on 46 breast cancer cases diagnosed 11 (S.D. 4) years (median 12, range 1–17) after entry into the study cohort. A reference sample of 138 women was thus drawn from the study base.

Among the referents were 3 breast cancer cases. These cases were excluded from the reference group in the multivariate analyses. The distribution among cases and among referents of the different variables included in the study is given in Table 1. Information on menopausal status of the cases at the time of diagnosis was not available.

Plasma selenium level in the base sample was 103 (22) μg/l and in the cases 109 (28) μg/l (95% confidence interval for the difference, −2 to 14 μg/l). The distributions of plasma selenium levels are given in Fig. 1. Bivariate analyses showed only weak associations between plasma selenium concentrations and age, age at menarche, age at first baby, parity and body mass index. Nevertheless, these variables have been adjusted for in the regression analyses.

As given in Table 2, analyses of the crude data indicated no association between plasma selenium levels and breast cancer risk. The risk estimates changed only slightly in multiple logistic regression analyses indicating no major confounding from the variables included in the model. Comparable results were obtained when the analyses were restricted to cases diagnosed more than 10 years after collection of blood samples.

DISCUSSION

In the present study selenium was not a strong indicator of human breast cancer risk. However, only a limited range of selenium exposures and a restricted time span were considered.

As already mentioned, there is no agreement between the

Table 2. Selenium distribution among cases and referents: all cases

Quartiles (μg Se/l)	Cases	Referents	OR	Adjusted OR (95% CI)†
>85	10	27	0.93	0.80 (0.29–2.19)
85–100	10	32	0.78	0.79 (0.30–2.09)
100–115	12	41	0.73	0.72 (0.28–1.82)
115<	14	35	1.00*	1.00

*Plasma selenium of more than 115 μg/l used as reference level.
†Adjusted for age, age at menarche, age at first baby, parity and body mass index.
OR = odds ratio.

results of previous case-referent studies. The study of McConnell *et al.* concerned plasma selenium levels in breast cancer patients with relatively advanced disease [3]. In an unpublished case-referent study we demonstrated an internal trend among breast cancer cases, which was in agreement with other studies [10], and the results of McConnell *et al.* may therefore be explained by disease-mediated changes in selenium levels.

The results of Schrauzer *et al.* are difficult to interpret as the paper includes only minor information on the cases and the referents included in the study [4]. However, an approximately 30% reduction in selenium levels among Japanese breast cancer cases is considerable, compared to the results of other studies.

In the present study, no major changes in the risk estimates were observed when the statistical evaluation was restricted to cases diagnosed more than 10 years after collection of blood samples. Disease-mediated changes in the observed risk estimates therefore seem unlikely.

In the study of Meyer and Verreault concerning erythrocyte selenium levels measured in cancer patients at least 6 months after diagnosis and treatment [5], knowledge about their disease most likely induced changes in the lifestyles of the women with breast cancer. Therefore, without information about changes in dietary habits, self-medication with dietary supplements and other changes in lifestyle, this study is non-informative.

Evidence from prospective studies have also been equivocal. Van Noord *et al.* found no importance for selenium in mammary carcinogenesis when prevalent and incident breast cancer cases were considered in a cohort of premenopausal women volunteering for breast cancer screening [7]. Selenium exposure was evaluated by measurements of nail selenium levels. Data on blood selenium levels from other studies indicated selenium levels in the Netherlands comparable to the levels measured in the present study [11]. Van Noord *et al.* demonstrated no difference in selenium levels between prevalent breast cancer cases and incident cases.

In a relatively small study including 20 breast cancer cases, Coates *et al.* demonstrated a positive association between serum or plasma selenium levels and breast cancer risk [6]. The levels of selenium were very high compared to the levels considered in previous studies. The confidence intervals were wide due to the few cases included in the study, but the consistent increase in cancer risk warrants further investigation.

In summary, the epidemiological evidence pertaining to an association between selenium and development of human breast cancer is still sparse. Case-referent studies are probably inconclusive, and the possibility of disease-mediated changes in selenium levels advocates for a prospective study design in the future.

The prospective studies conducted so far, including the present study, have indicated no importance of selenium in human mammary carcinogenesis when moderate levels of selenium exposure were considered. The study of Coates *et al.* indicated an unexpected positive association between selenium and breast cancer risk [6]. As a cancer promotive effect of selenium has also been demonstrated in some animal experiments [12, 13], the results of Coates *et al.* should be explored further in new studies conducted in areas of the world where high levels of selenium intake occur.

1. Shamberger RJ, Tytko SA, Willis CE. Antioxidants and cancer. Part IV. Selenium and age-adjusted human cancer mortality. *Arch Environ Health* 1976, **31**, 231–235.
2. Schrauzer GN, White DA, Schneider CJ. Cancer mortality correlation studies—III: Statistical associations with dietary selenium intakes. *Bioinorg Chem* 1977, **7**, 23–34.
3. McConnell KP, Jager RM, Bland KI, Blotcky AJ. The relationship of dietary selenium and breast cancer. *J Surg Oncol* 1980, **15**, 67–70.
4. Schrauzer GN, Molenaar T, Mead S, Kuehn K, Yamamoto H, Araki E. Selenium in the blood of Japanese and American women with and without breast cancer and fibrocystic disease. *Jpn J Cancer Res (Gann)* 1985, **76**, 374–377.
5. Meyer F, Verreault R. Erythrocyte selenium and breast cancer risk. *Am J Epidemiol* 1987, **125**, 917–919.
6. Coates RJ, Weiss NS, Daling JR, Morris JS, Labbe RF. Serum levels of selenium and retinol and the subsequent risk of cancer. *Am J Epidemiol* 1988, **128**, 515–523.
7. Van Noord PAH, Collette HJA, Maas MJ, De Waard F. Selenium levels in nails of premenopausal breast cancer patients assessed prediagnostically in a cohort-nested case-referent study among women screened in the DOM project. *Int J Epidemiol* 1987, **16** (Suppl.), 318–322.
8. Hertel N, Thorlacius-Ussing O. PIXE analysis of biomedical samples. *Nucl Instr Meth* 1987, **B22**, 149–151.
9. Johansson GI. Modifications of the HEX program for fast automatic resolution of PIXE-spectra. *X-Ray Spectrom* 1982, **11**, 194–200.
10. Robinson MF, Godfrey PJ, Thomson CD, Rea HM, Van Rij AM. Blood selenium and glutathion peroxidase activity in normal subjects and in surgical patients with and without cancer in New Zealand. *Am J Clin Nutr* 1979, **32**, 1477–1485.
11. Thorling EB, Overvad K, Geboers J. Selenium status in Europe–human data. A multicenter study. *Ann Clin Res* 1986, **18**, 3–7.
12. LeBoeuf RA, Laishes BA, Hoekstra WG. Effects of selenium on cell proliferation in rat liver and in mammalian cells as indicated by cytogenetic and biochemical analysis. *Cancer Res* 1985, **45**, 5496–5504.
13. Birt DF. Update on the effects of vitamin A, C, and E and selenium on carcinogenesis. *Proc Soc Exp Biol Med* 1986, **183**, 311–320.

Acknowledgement—The present study was supported by Helsefonden and by the Danish Cancer Society.