Serum iodine and breast cancer risk: A prospective nested case-control study stratified for selenium levels

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Abstract

Background: Iodine has been suggested to protect against breast cancer, but there are no epidemiological studies on individual risk. An interesting finding is that in areas where the exposure to both selenium and iodine are high (e.g. Japan), the risk of breast cancer is lower than in areas where selenium is high and iodine low (e.g. USA), or in areas where both are low (e.g. Northern Europe). The aim of this study was to investigate the association between pre-diagnostic serum iodine levels and subsequent breast cancer risk, and to investigate if this potential association was modified by selenium levels.

Methods: The Malmö Diet and Cancer Study provided pre-diagnostic serum samples and the current analysis included 1159 breast cancer cases and 1136 controls. Levels of baseline serum iodine and selenium were analyzed. A logistic regression analysis yielded odds ratios (OR) with 95% confidence intervals adjusted for potential confounders.

Results: There was no evidence of an overall association between iodine levels and risk of breast cancer. Among women with high selenium levels (above the median), high iodine levels were associated with a lower risk of breast cancer; the OR for above vs. below the median was 0.75 (0.57-0.99). The corresponding OR for women with low selenium was 1.15 (0.87-1.50), and the p-value for interaction was 0.06.

Conclusions: The combination of high serum iodine levels and high selenium levels was associated with a lower risk of breast cancer.

Impact: A high iodine and serum exposure may decrease the risk of breast cancer.

Word count: 249

Introduction

Iodine has been suggested to protect against breast cancer, but there are no epidemiological studies on individual risk. High iodine intake has been proposed to lead to a low risk of breast cancer in Japanese women (1,2,3), and an early correlation study indicated that a high iodine intake is protective (4). A study from Spain found an inverse association between iodine intake in different geographical areas and breast cancer mortality (5), but there have been no studies on iodine levels in individual women and breast cancer risk. Interestingly, a recent meta-analysis reported that women treated with radioactive iodine (RAI) for thyroid cancer had a relative risk of breast cancer of 0.61, as compared to thyroid cancer patients not treated with RAI (6).

There is strong biological evidence of a potential protective effect from iodine regarding breast cancer. Iodine receptors, such as the sodium/iodide symporter (NIS), Pendrin, and the sodium/moncarboxylate transporter (SMCT), are present in breast tissue, which enables uptake of iodine (7). Iodine is necessary for normal breast development and iodine deficiency in rats cause breast atypia and dysplasia, which are reversible with iodine supplementation (8,9). Iodine has also been proposed to act as an antioxidant, to have antiproliferative effects and to stimulate apoptosis in breast tissue (3,10,11).

Iodine metabolism is closely related to selenium, e.g. in the regulation of thyroid hormones, and selenium had also been implicated as a protective factor for breast cancer (9). Two early reviews (including 17 studies) reported no association between selenium and breast cancer risk (9,12), and later, this was confirmed by a Cochrane analysis (13). Following this, we performed a study including more than 1000 cases, confirming the findings in previous reviews (14). An interesting finding is that breast cancer mortality in The US (on average low iodine and high selenium levels) and Europe (low iodine and low selenium) is about 4-5 times higher than in Japan where both selenium and iodine are high (2,9,15).

The aim of this study was to investigate the association between pre-diagnostic serum iodine levels and subsequent breast cancer risk, and to investigate if this potential association is modified by selenium levels.

Materials and methods

The Malmö Diet and Cancer study (MDCS)

The MDCS invited all men and women who were born between 1923 and 1950 and lived in Malmö; Sweden. Between 1991 and 1996, a total of 17,035 women were recruited, corresponding to a participation rate of 41%. The baseline examination included a questionnaire on socio-demographic data, life-style, reproductive factors, and medications. Everyone was measured concerning height and weight. Blood samples were drawn at baseline and they have been stored at -80°C (16). The questionnaire assessed most risk factors for breast cancer and menopausal status was constructed using information on menstrual bleedings and previous gynecological surgery (14). A dietary assessment was performed through a modified dietary history method, using a 168-item dietary questionnaire, a 7-day menu book, and a one-hour diet history interview (17).

The present study was approved by the regional ethical committee (Dnr 2015/283), and all participant signed a written informed consent at baseline in order to allow collection of information and samples, as well as future follow-up (original ethical approval: LU 51-90).

Matching of cases and controls

Breast cancer cases, both invasive and cancer *in situ*, were identified by record linkage with The Swedish Cancer Registry up until the end of follow-up; 31 December 2013. There were 576 women who had been diagnosed with breast cancer prior to baseline examination and they were excluded from the present analysis. The 1186 women who had been diagnosed with

breast cancer after baseline examination were considered as incident cases and they were included in the present study. Cancer cases were described with regard to invasive / *in situ* status and the expression of hormone receptors. Estrogen receptor (ER) expression for cases in this cohort was assessed using immunohistochemistry and this has previously been described in detail (18). For cases diagnosed up until 2004, this information was obtained by re-analyzing original tumor samples. Henceforth, this information was available from clinical notes and routine pathology reports. The cut-off for negative/positive status was 10%.

The selection of controls was based on two demands; to use a previous case-control data set with additional important information, and the need to add new cases and controls, with the possibility to add information on genetic factors. Selection of the first set of controls in the MDCS was based on a previous study examining the association between breast cancer and vitamin D. That study included cases diagnosed up to 31 December 2005 (n=764) and an equal number of controls identified using incidence density matching using age, menopausal status and time of baseline examination as matching factors (19). Using incidence density matching, some individuals were used more than once, as control/case or as control/control. The selection resulted in 704 unique control individuals out of whom 694 remained free of breast cancer up until 31 December 2013. The second set of controls were selected from the MDCS cardio-vascular cohort to get the same number of controls as the 1186 cases. This sub-cohort includes 3531 randomly selected women from the total MDCS cohort examined 1991-94. Of these, 2615 were complete participants in MDCS without breast cancer up until 31 December 2013, and without being used previously as controls. In this group, 492 were randomly selected to get a total of 1186 controls. The reason to select controls from the MDCS cardio-vascular cohort was that they were planned to be genotyped.

Laboratory analyses

Serum analyses were performed by ALS Scandinavia AB, Luleå, Sweden, on samples collected at baseline, which had been stored at -80°C. Serum samples were analyzed by using single element standards, traceable to NIST, on ICP-SFMS (Thermo Element 2). An amount of 0.15 ml serum was diluted to 10ml with an alkalic solution containing 0.1% NH3 and 0.005% EDTA/Triton-X. Reference material, Seronorm, was obtained from Sero AS, Norway (Lot 0608414) and two reference samples were added in each batch. CV for inter-batch variation was 0.04 for iodine and 0.03 for selenium. Samples were analyzed in the order they had been collected, not related to case-control status. Following the initial analysis, 77 samples showed very high or extreme iodine levels, i.e. above 1000 µg/L. Following re-analysis of 14 samples confirming these levels, it was concluded that these high values were most likely caused by contamination when blood samples were drawn. It was, hence, decided to exclude these 77 individuals (27 cases and 50 controls) from further analysis, this is described in detail in Supplementary Methods and Materials. Some individuals (130 cases and 132 controls) had insufficient amount of biological material or the analysis failed.

Statistical analysis

Out of all 2372 individuals (1186 cases and 1186 control), 77 had extreme iodine values (see above) and they were excluded. Individuals with missing information on iodine levels were, however, kept and reported as a separate category. Hence, the final study population consisted of 1159 cases and 1136 controls.

The cohort was divided into quartiles based on serum iodine levels, for both cases and controls together, and with specific cut-offs for separate single baseline years (see Supplementary Methods and Materials). Selenium was handled in the same way using year-specific cut-offs in order to dichotomize the cohort into high (above the median) or low

(below the median) selenium levels. Iodine quartiles were compared regarding established and potential risk factors for breast cancer, and for factors that may influence serum iodine/selenium levels. Potential differences were tested using a Chi-2 test (excluding missing categories). An unconditional logistic regression analysis was used to obtain odds ratios (OR) with 95% confidence intervals for different quartiles, as compared to the first. Linear trends over quartiles was assessed, using the median value for each quartile in the model. A second model included established and potential risk factors for breast cancer, and factors that may influence serum iodine/selenium levels, i.e. age at baseline, socio-economic status, education, marital status, number of children, age at first childbirth, age at menarche, oral contraceptives, menopausal status, oophorectomy, hormone replacement therapy, smoking status, BMI, alcohol consumption, and the month samples were collected. All factors were entered as categorical variables. Time between baseline examination and analysis, i.e. storage time, was already accounted for by using year-specific cut-off levels. Missing values for co-variates were handled as a separate category. Missing was a minor problem concerning included co-variables, most factors having less than 2% of individuals with missing information. ORs were reported for the group with no information on iodine levels in order to examine if there was a systematic pattern concerning missing.

The analyses were also performed stratified for high/low selenium levels. Following quartile analyses, a threshold effect was suggested, and analyses were also performed using iodine as a dichotomized factor. An interaction term for iodine quartile (high/low category) and selenium category was included in the logistic regression model and tested using the Wald statistic.

Finally, we performed several sensitivity analyses concerning the main findings, i.e. iodine levels analyzed as a dichotomized factor and stratified for selenium levels. The first analysis excluded all cases who got breast cancer within two years from baseline examination.

The second analysis excluded *in situ* cases (n=98), and a third analysis was stratified for ER-status. A fourth analysis included potential confounders related to diet that has been suggested as potential risk factors for breast cancer, i.e. vitamin D, beta-carotene and calcium (20). Dietary factors were classified into quintiles and the model was also adjusted for total energy intake. A set of sub-group analyses were explorative and not based on an à priori hypothesis. Hence, a fifth analysis was made separately for pre-/perimenopausal and postmenopausal women. A sixth model was stratified for age at diagnosis and we used 55 years of age at a cut-off as we previously had used this cut-off to define postmenopausal status where other information was missing (19). Older women were divided into 55-65 and above 65 years of age, given that median age at diagnosis was 65.9 years. Samples were taken before diagnosis and a final sensitivity analysis dichotomized cases according to time between baseline and time of diagnosis, using the median. All p-values were two-sided and a p-value less than 0.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS v24.0.

Results

Iodine serum levels were slightly higher in older women. Other factors statistically significantly associated with iodine levels were menopausal status, use of HRT, and alcohol consumption, table 1.

There was no statistically significant overall association between iodine levels and risk of breast cancer, table 2. Among women with high selenium levels (above the median), high iodine levels were associated with a lower risk of breast cancer; the adjusted OR for above vs. below the median was 0.75 (0.57-0.99). The quartile analysis showed a similar pattern, with a relatively low risk in quartile 3 and 4 as compared to the 1st, but these associations were not statistically significant. There were no corresponding associations among women with low

selenium levels. The p-value for interaction, comparing the iodine associated risk in women with high vs. low selenium level was 0.06 (0.04 in the crude analysis), table 2.

In the sensitivity analysis excluding cases during the first two years following baseline, all ORs were very similar to the main analysis, supplementary table 1. The same was observed when *in situ* cases were excluded. The analysis including only ER-positive cases was similar to the main analysis but the analysis on ER-negative cases showed ORs close to unity and broad confidence intervals. All ORs were also similar when the analyses were adjusted for dietary factors, supplementary table 1. Menopausal status at baseline did not seem to affect the overall association between iodine and breast cancer. If anything, the negative association in women with high selenium levels was slightly stronger among postmenopausal women, supplementary table 2. Age at diagnosis and time between baseline and breast cancer diagnosis co-varies. In women with low selenium levels, there was a statistically significant positive association between high iodine levels and breast cancer for cases diagnosed relatively early. If anything, the negative association between iodine and breast cancer among women with high selenium levels was somewhat stronger in women diagnosed later during follow-up or at high age, supplementary table 2.

Discussion

Our study found that among women with high selenium levels, high iodine levels were associated with a risk of breast cancer that was about 25% lower than among women with low iodine levels. This is in line with the hypothesis that the combination of high iodine and high selenium levels, as seen in Japanese women, can be related to a low risk of breast cancer (2,9).

Our descriptive analysis found that age, post-menopausal status, use of HRT and a relatively modest alcohol consumption was positively associated with iodine levels. Age,

menopausal status and HRT are tightly related. Moreover, in the current cohort, older women drink less alcohol (data now shown). There are, to our knowledge, a very small number of previous studies on determinants for iodine status, but a recent study has reported that serum iodine increases with age (21), and this is in line with our study.

This is the first study ever on the association between serum iodine levels and breast cancer risk using individually measuring iodine levels among cases and controls. Our study is, moreover, prospective using pre-diagnostic serum iodine levels. Previous studies comparing incidence rates of breast cancer with iodine status in different populations have suggested a protective effect from iodine (1,2,4,9). An ecological study from Spain found an inverse association between iodine intake in different geographical areas and corresponding breast cancer mortality (correlation coefficient 0.44). The study found that areas with a low iodine status had a relative risk of 1.87 relating to breast cancer mortality as compared to high iodine areas (5). Our study did not, however, find an overall association between iodine levels and breast cancer risk.

Results from studies of women who have undergone radioactive iodine (RAI) treatment for thyroid disease are problematic to interpret as the potential association between iodine and breast cancer risk may be caused by confounding by indication, non-physiological doses, and selection of study participants. However, they may offer some indirect evidence. The meta-analysis mentioned above reported that women treated with RAI for thyroid disease have a decreased risk of breast cancer (6). This was also found in a study by Ahn et al (22) and in a study by Kuo et al (23), however, in the former study only statistically significant following high-dose treatment (hazard ratio 0.17 for RAI vs. not), and in the later only statistically significant in the univariable analysis.

Some methodological issues have to be considered. Serum samples had been stored for about 20 years and it is not known to what extent iodine levels may have changed during

storage e.g. due to degradation or evaporation of the sample. We handled this by using separate cut-point for different calendar years. The inter-batch coefficient of variation for the iodine analysis was 0.04, which strengthens the reliability of our measurements. Concerning our endpoint, incident breast cancer, The Swedish Cancer Registry was used to identify cases, a registry with a high validity and completeness (24). The participation rate in the MDCS was 41%, but previous analyses have shown that participants are similar to the overall background population regarding factors like socio-economy, BMI and smoking (16) and this increase the generalizability of the results. A weakness of our study design is that we used two different types of selection criteria for our control group; some were matched and some were randomly selected. However, matching factors was adjusted for in the analyses, which ought to have limited this potential problem.

Several sensitivity analyses were performed. There was nothing to suggest that sub-clinical disease had affected the results, given similar results when cases diagnosed within two years following baseline were excluded. Similarly, all results were very similar when *in situ* cases were excluded, when the analysis only included ER-positive cases, and when the model was adjusted for dietary factors. The analysis in relation to ER-negative cases included few cases and had a limited statistical power. We had no à priori hypothesis on a potential modifying effect of menopausal status, age at diagnosis or time of follow-up. Only including cases diagnosed during the first half of the follow-up period, the finding of a positive association between iodine and breast cancer risk in women with low selenium levels was not expected given the hypothesis. Due to the large number of additional analyses, a type I error is difficult to exclude.

Iodine receptors such as NIS and Pendrin are expressed during lactation and in breast cancer, but are not seen in normal breast tissue. On the other hand, SMCT is seen in normal breast tissue but not in breast cancer (7). During lactation, iodine is incorporated in the breast

into iodoproteins and iodolipids which have been suggested to have antiproliferative effects (9). There is also some evidence that iodine *per se* may act as an antioxidant (10), and that iodine may have antiproliferative and apoptotic effects (3,11). Interestingly, the iodine receptor SMCT function as a tumor suppressor (7), which may delay tumor progression. That iodine receptors may affect breast cancer development is indirectly suggested by the finding that metastatic breast cancer has relatively low levels of NIS (25).

Considering breast tissue specifically, experimental studies have shown that iodine deficiency leads to dysplasia and the gland becomes highly sensitive to estradiol (8,26), factors that would increase cancer risk. Indeed, Kilbane et al reported that breast tumors had a lower tissue content of iodine as compared to normal tissue (27). Experimental studies have also shown that iodine, e.g. in seaweed, given to rats with breast cancer leads to a reduction in tumor size and delays tumor development (28). In line with this, seaweed also induce apoptosis in human breast cancer cell lines (29).

Selenium in relation to breast cancer has been studied in a large number of studies, showing not clear association with risk (9,12,13). However, there are well-known biological mechanism that in theory may lead to a protective effect in relation to breast cancer. Selenium exerts many important effects through the action of selenoenzymes, many of which are potent antioxidants. They may also regulate transcription, cell proliferation, apoptosis. and selenium may also improve the immune response (15). Concerning selenium, it has been suggested that it is the combination with high iodine that may lower the risk of breast cancer (2,9). This hypothesis is mainly based on ecological studies comparing different geographical areas, often using Japan as an example. If true, there are several reasons why women with a combination of high iodine and high selenium levels have a decreased risk of breast cancer. It may simply be an additive effect that leads to an effect large enough to be observed. Considering serum selenium, we found no association with risk and this is in line with a

previous Cochrane review (13,14). For iodine, there are no previous case-control or cohort studies, and the current study did not support an overall risk. Given the results in the current study, it is possible that there is a true biological interaction between iodine and selenium, but the mechanisms are not clear. It has been suggested that iodine deficiency leads to changes in the breast such as atypia, dysplasia and even neoplasia. Low iodine levels also seem to increase the sensitivity to estrogens (1, 9). Such factors may not necessarily increase the risk of breast cancer, but in the absence of a protective effect from selenium this combination may define women with a high risk, i.e. high iodine in combination with high selenium may be protective. Future experimental studies are needed to investigate this further.

Following that a combination of high iodine/selenium was associated with a 25% lower risk of breast cancer, we conclude that it is important to further investigate if the combination of high iodine and selenium levels may be protective with regard to breast cancer. An important aspect is that our study is the first on this topic, and the result has to be replicated.

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Table 1. Established and potential risk factors for breast cancer in relation to quartiles (Q) of serum iodine levels.

Factor	Category	Q1 (n=506)	Q2 (n=509)	Q3 (n=512)	Q4 (n=506)	p-value	Missing (n=262) %
		Column %					Column %
Age	≤50	25.7	25.5	22.7	20.6	p<0.001	13.4
	>50-≤55	29.1	24.6	25.4	18.0		16.4
	>55-≤60	20.0	20.8	18.8	21.7		17.9
	>60	25.3	29.1	33.2	39.7		52.3
Socio-economic index	Manual	33.6	36.3	36.1	37.5	p=0.79	38.9
	Non-manual	60.1	56.2	55.7	54.7		56.9
	Employer	5.9	6.5	6.4	7.1		3.8
Education	≤9 years	62.3	67.4	70.1	69.2	p=0.13	79.4
	10-12 years	9.7	6.7	6.8	7.1		6.5
	University	27.9	25.5	23.0	23.3		14.1
Married or cohabiting	No	32.0	33.6	30.5	35.0	p=0.45	29.8
	Yes	68.0	66.4	69.5	65.0		70.2
Parity	0	13.4	13.9	13.7	12.8	p=0.79	12.2
	1	19.6	18.7	20.9	19.6		22.5
	2	43.1	45.0	42.4	43.5		40.1
	3	15.4	14.7	17.8	15.2		17.2
	≥4	5.5	5.9	3.1	5.5		5.3
	Missing	3.0	1.8	2.1	3.4		2.7
Age at first childbirth	≤20	17.2	15.5	16.2	17.2	p=0.61	18.3
	>20-≤25	31.6	33.4	37.1	36.0		35.1
	>25-≤30	23.9	27.1	22.9	21.1		22.9
	>30	10.7	8.3	8.0	9.5		8.8
	Nulliparous	13.4	13.9	13.7	12.8		12.2
	Missing	3.2	1.8	2.1	3.4		2.7
Age menarche	≤12	21.9	23.0	23.8	20.9	p=0.85	21.0
	>12-≤14	53.0	51.3	52.1	52.8		54.6
	>14	24.3	25.3	22.7	26.3		22.5
Ever use of oral contraceptives	No	44.9	46.4	52.1	50.0	p=0.08	53.8
	Yes	55.1	53.6	47.9	50.0		45.8
Menopausal status	Pre	30.6	30.8	25.8	22.7	p<0.001	15.3
	Peri	11.3	9.0	7.8	6.7		3.8
	Post	58.1	60.1	66.4	70.6		80.9
Oophorectomy. bilateral	No	98.6	98.4	98.8	97.8	p=0.61	98.1
	Yes	1.4	1.6	1.2	2.2		1.9
HRT, use at baseline	No	79.6	81.1	74.8	70.8	P<0.001	80.9
	Yes	20.2	18.5	25.0	29.1		18.7
Smoker	Never	46.0	41.8	42.2	40.7	p=0.51	45.4
	Current	27.5	28.3	26.4	28.1		26.7
	Ex	26.3	29.9	31.4	31.2		27.9
BMI	<20	4.5	4.3	5.5	6.5	p=0.33	3.8
	≥20-<25	52.4	47.9	45.9	46.6		42.7
	≥25-<30	33.8	34.6	35.4	34.2		34.7
	≥30	9.3	13.2	13.3	12.5		18.7
Alcohol consumption	Zero	3.8	5.5	7.4	9.5	p<0.001	8.8
	<15g/day	62.1	64.8	62.7	64.4		64.5
	15-30g/day	19.2	16.5	12.1	10.7		10.7
	>30g/day	4.7	2.6	2.9	3.2		3.4
	Infrequent	10.3	10.6	14.6	12.3		12.6

Missing data not presented if a factor had <2% missing in all quartiles.

Table 2. Iodine levels and breast cancer risk, overall and stratified for selenium levels.

Iodine level	Iodine[2] (µg/L)	All			Low selenium[2] (≤92.0 µg/L)			High selenium[2] (≥88.0 µg/L)				
		Cases/ Controls	OR (95 CI)	OR[1] (95 CI)	Cases/ Controls	OR (95 CI)	OR[2] (95 CI)	Cases/ Controls	OR (95 CI)	p_i	OR[2] (95 CI)	p_i
Q1	≤61.6	254/252	1	1	155/164	1	1	99/88	1		1	
Q2	58.1-70.9	271/238	1.13 (0.88-1.45)	1.16 (0.90-1.50)	135/124	1.15 (0.83-1.60)	1.15 (0.82-1.64)	136/114	1.06 (0.73-1.55)	*0.75*	1.06 (0.70-1.59)	*0.64*
Q3	65.5-82.2	253/259	0.97 (0.76-1.24)	0.97 (0.75-1.26)	124/118	1.11 (0.80-1.55)	1.20 (0.83-1.72)	129/141	0.81 (0.56-1.18)	*0.22*	0.78 (0.52-1.17)	*0.19*
Q4	≥73.2	251/255	0.98 (0.76-1.25)	1.01 (0.78-1.30)	106/88	1.27 (0.89-1.82)	1.26 (0.86-1.85)	145/167	0.77 (0.54-1.11)	*0.05*	0.78 (0.52-1.16)	*0.08*
	Trend *p-value*	1029/1004	*0.57*	*0.68*	520/494	*0.21*	*0.24*	509/510	*0.06*	--	*0.10*	--
	Missing vs Q1	130/132	0.98 (0.72-1.32)	1.01 (0.74-1.39)	--	--	--	--	--	--	--	--
Low	≤70.9	525/490	1	1	290/288	1	1	235/202	1		1	
High	≥65.6	504/514	0.92 (0.77-1.09)	0.92 (0.76-1.10)	230/206	1.11 (0.87-1.42)	1.15 (0.87-1.50)	274/308	**0.76 (0.60-0.98)**	***0.04***	**0.75 (0.57-0.99)**	*0.06*

[1] Adjusted for age at baseline, socio-economic status, education, marital status, parity, age at first childbirth, age at menarche, oral contraceptives, menopausal status, oophorectomy, hormone replacement therapy, smoking status, BMI, alcohol consumption, and calendar month samples were collected. All entered as categorical variables.
[2] Categorisations of quartiles (Q) and medians were year-specific and absolute values may overlap. The year-specific median was used to classify selenium as low/high.
p_i is the p-value in the interaction analysis comparing association between iodine levels and breast cancer risk in women with high vs. low selenium levels.
Some women had no data on selenium levels why numbers in stratified analyses do not equal the total.

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Serum iodine and breast cancer risk: A prospective nested case-control study stratified for selenium levels

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