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Pre-diagnostic acrylamide exposure and survival after breast cancer among postmenopausal Danish women

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ABSTRACT

Acrylamide is a probable human carcinogen, with industrial contact, tobacco smoking and foods processed at high temperatures as the main routes of exposure. In animal studies oral intake of acrylamide has been related to cancer development, with indications that the increased cancer occurrence especially regards endocrine related tumors. In human epidemiological studies, dietary exposure to acrylamide has also been suggested related to higher risk of endocrine related tumors, like estrogen sensitive breast cancer. The aim of the present study was to evaluate if pre-diagnostic acrylamide exposure, measured by acrylamide and glycidamide hemoglobin adducts (AA-Hb and GA-Hb), were associated to mortality in breast cancer cases. Among 24,697 postmenopausal women included into a Danish cohort between 1993 and 1997, 420 developed breast cancer before 2001 and 110 died before 2009. AA-Hb and GA-Hb concentrations measured in blood samples were related to mortality by Cox proportional hazard models. Estimates are given per 25 pmol/g globin higher levels.

Among non-smokers, higher concentrations of GA-Hb were associated to a higher hazard rate of breast cancer specific mortality (HR (95% CI): 1.63 (1.06–2.51)), the hazard rate among women diagnosed with estrogen receptor positive tumors was (HR (95% CI): 2.23 (1.38–3.61)). For AA-Hb the tendency was similar, but only statistically significant among those with estrogen receptor positive tumors (HR (95% CI): 1.31 (1.02–1.69)). In conclusion, the present study indicates that pre-diagnostic exposure to acrylamide may be related to mortality among breast cancer patients and that this may especially concern the most endocrine related type of breast cancer.

1. Introduction

Acrylamide is classified as a probable human carcinogen (class 2A) (IARC, 1994). Industrial contact with acrylamide was originally the main concern with regard to acrylamide exposure, but more recently it has been established that tobacco smoking (Urban et al., 2006) and processing of foods at high temperatures (Tareke et al., 2000) are probably more important routes of exposure for a large majority of the population.

The human metabolism of acrylamide follows two main pathways: conjugation with glutathione or epoxidation. Acrylamide is oxidized to the epoxide glycidamide. Cytochrome P450 2E1 is the primary enzyme responsible for the epoxidation. Glycidamide itself is further metabolized through conjugation with glutathion or hydrolysis to 2,3-dihydroxy-propionamide (Calleman et al., 1990; Doroshyenko et al., 2009; Ghanayem et al., 2005; Hartmann et al., 2010; Sumner et al., 1999). Both acrylamide and glycidamide are reactive compounds that form adducts with proteins, including hemoglobin. Contrary to acrylamide, glycidamide is mutagenic and is generally considered the causative genotoxic metabolite of acrylamide (Besaratinia and Pfeifer, 2007; Paulsson et al., 2001).

The concentration of acrylamide bound to the N-terminal amino acid in hemoglobin is strongly correlated to the exposure of acrylamide. The glycidamide analog correlates to glycidamide DNA adducts (Tareke et al., 2006) and is considered a biomarker for the genotoxic dose reflecting the individual ability to metabolically activate acrylamide. Adducts formed by acrylamide and glycidamide with hemoglobin (AA-Hb and GA-Hb) are considered good

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measurements of an individual's average exposure to acrylamide and glycidamide within the 4 months life-time of an erythrocyte (Bergmark, 1997; Paulsson et al., 2003).

In animal studies, acrylamide has been reported carcinogenic following oral dosing. Looking at the cancer sites evaluated in the published rat studies, there are indications that especially the occurrence of endocrine related tumors are increased following acrylamide exposure (Friedman et al., 1995; Johnson et al., 1986).

In human epidemiological studies, the association between dietary exposure to acrylamide and risk of cancer development has been evaluated in several studies, recently reviewed by Hogervorst et al. (2010). Some of the published epidemiological studies seem to support that acrylamide primarily is associated with endocrine related cancer, as the strongest associations have been found for endometrial and ovarian cancer as well as estrogen and/or progesterone receptor positive breast cancer (Hogervorst et al., 2007; Olesen et al., 2008; Pedersen et al., 2010; Wilson et al., 2010). Other studies evaluating the same cancer sites have, however, not found associations with acrylamide exposure and consequently do not agree with the hormone hypothesis (Hogervorst et al., 2010). Further, the biological mechanisms behind the potential effect on endocrine related cancer are not elucidated, but it has been suggested that acrylamide may influence the hormonal balance through binding to the estrogen receptor (Hogervorst et al., 2010).

Circulating estrogen levels and the binding of estrogen to the estrogen receptor are known to be among the most important risk factors for breast cancer and influence on circulating estrogen levels is the key mechanism behind several of the factors known to be associated to breast cancer incidence and/or prognosis (Folkerd and Dowsett, 2010; Kendall et al., 2007). Special concern has been given to the impact of obesity in postmenopausal women, where estrogens produced through aromatization in adipose tissue result in significantly higher concentrations of circulating estrogens in obese women compared to lean women (Liedtke et al., 2012).

If acrylamide and/or glycidamide affect the hormonal environment in women it is possible, that exposure to high levels of acrylamide is also related to a poorer prognosis after breast cancer diagnosis.

The aim of the present study was to evaluate if pre-diagnostic acrylamide exposure, measured by AA-Hb and GA-Hb, was related to mortality among 420 postmenopausal women diagnosed with breast cancer. This hypothesis has, to our knowledge, not previously been studied in epidemiological studies.

2. Materials and methods

2.1. Cohort

The Diet, Cancer and Health study is a prospective cohort study, established with the primary aim of studying the etiological role of diet on cancer risk. Between 1993 and 1997, 79,729 Danish women aged 50–64 and without previous cancer diagnoses were invited to participate in the study. A total of 29,875, corresponding to 37% of those invited, were enrolled into the cohort. A detailed description of the cohort, including socioeconomic factors associated to non-response has been published previously (Tjonneland et al., 2007).

The Diet, Cancer and Health study and the present substudy were approved by the regional ethical committees on human studies in Copenhagen and Aarhus and by the Danish Data Protection Agency. All participants provided written informed consent.

All cohort members attained one of the two established study centers, and each participant filled in a food frequency questionnaire and a life-style questionnaire. The life-style questionnaire included questions about reproductive factors, health status, social factors, and life-style habits. From this questionnaire, we obtained information about use of hormone replacement therapy (HRT; use at baseline yes/no), smoking at baseline (yes/no), smoking duration (years) and tobacco use (g/day). Information about alcohol intake (g/day) was obtained from the food frequency questionnaire.

In the study centers, 30 mL of blood (nonfasting, collected in citrated and plain Venojects) were drawn from each participant. The samples were spun and divided into 1-mL tubes of plasma, serum, erythrocytes, and buffy coat. All samples were processed and frozen within 2 h at −20 °C. At the end of the day of collection, all samples were stored in liquid nitrogen vapor (maximum temperature, −150 °C).

Of the initial 29,875 women, we excluded 326 who later were reported to the Danish Cancer Registry with a cancer diagnosed before the visit to the study clinic. In addition, eight women were excluded from the study because they did not fill in the life-style questionnaire. Because the present analysis aimed at women who were postmenopausal at study entry, we further excluded 4844 women, including 4798 who were considered premenopausal because they had reported at least one menstruation <12 months before entry and no use of HRT, nine women who gave a lifetime history of no menstruation, and 37 women who did not answer the questions about current or previous use of HRT, leaving 24,697 postmenopausal women for study.

Cohort members were identified from their unique personal identification number, which is allocated to every Danish citizen by the Central Population Registry. All the postmenopausal cohort members were linked to the Central Population Registry to obtain information on vital status and emigration. Information on cancer occurrence among cohort members was obtained through record linkage to the Danish Cancer Registry, which collects information on all cases of cancer diagnosed in Denmark (Storm et al., 1997). Linkage was done by the use of personal identification number and follow-up was nearly complete (99.8%). Each cohort member was followed up for breast cancer occurrence from date at entry, that is, date of visit to the study center until the date of diagnosis of any cancer (except for non-melanoma skin cancer), date of death, date of emigration, or December 31, 2000, whichever came first. Incident breast cancer was diagnosed in 434 women during the follow-up period. Of these 14 were excluded due to lack of blood sample or failure in the adduct analysis, leaving 420 breast cancer cases for study. The 420 women diagnosed with breast cancer before 1/1 2001 were linked to the Danish Death Certificate Registry to obtain information about date and cause of death. All cases (100% follow-up) were followed from date of diagnosis (between baseline and end 2000) until death or December 31, 2008.

A clinical registry exclusively about breast cancer also exists in Denmark and information on estrogen receptor α (ERα) status was obtained by linkage with the Danish Breast Cancer Co-operative Group, which holds records on a range of details for approximately 90% of all breast cancers diagnosed in Denmark (Fischerman and Mouridsen, 1988). A standardized immunohistochemical method was used in all medical centers. The cutoff level used to define positive ERα status was ≥10% positive cells. Information on ERα status was registered for 388 (92%) of the breast cancer cases, of these 299 women were diagnosed with an ERα positive tumor and 89 with an ERα negative tumor.

2.2. Adduct analysis

The analysis of the blood samples was conducted according to the method described in (Bjellaas et al., 2007). In short, the globin was purified from the blood samples and stored at −20 °C until analysis. Globin (20 mg) was subjected to a modified Edman reaction where phenyl isothiocyanate reacts with the N-terminal amino acid (valine) in hemoglobin, undergoes cyclization and decouples from the hemoglobin molecule releasing a phenylthiohydantoin derivative of N-alkylated valine adducts. The released phenylthiohydantoins were purified by solid phase extraction and analyzed on a LC ion-trap MS using multiple reaction monitoring.

The limit of quantification (LOQ) of the analysis was determined from the standard deviation of blanks (LOQ = 10 × SD/slope of calibration curve) to 2.4 pmol/g globin and 6.8 pmol/g globin for the AA-Hb and GA-Hb, respectively. In total, 42 batches were run. All samples were injected into the LC/MS in triplicate. All values were above the LOQ for AA-Hb but 8 values were below LOQ for GA-Hb.

The association between AA-Hb and GA-Hb concentrations and incidence of breast cancer has previously been reported based on these 420 breast cancer cases and a corresponding number of controls (Olesen et al., 2008).

2.3. Statistical methods

Analyses of the relations between AA-Hb and GA-Hb and mortality were based on Cox proportional hazard models using follow-up (from date of diagnosis) as the time axis and stratifying by age at diagnosis in 5 year intervals. All analyses were stratified on smoking status at baseline (yes/no), as tobacco smoking is an important source of acrylamide exposure. Former smokers and never smokers were pooled, as AA-Hb and GA-Hb concentrations were identical in these two groups. Special concern was given to recent former smokers (within 6 months of study entry), but their adduct levels did not differ from never smokers. To further elucidate influence of smoking on AA-Hb and GA-Hb levels, analyses conducted on smokers were adjusted for smoking duration (years) and tobacco use (g/day), both included as continuous variables. Analyses were also stratified according to the estrogen receptor status (positive or negative) of the tumor at diagnosis. Only 30% of the breast cancer cases were diagnosed with an estrogen receptor negative tumor, and when this minor sub-group was also stratified on smoking status, the statistical strength became very limited. Results are consequently only presented for either all breast cancer cases or for those with estrogen receptor positive tumors.

Factors previously found related to mortality among breast cancer patients were evaluated as potential confounders (Barnett et al., 2008; Carlsen et al., 2008; Dal et al., 2008). The associations are presented with and without adjustment for time

between blood drawn and breast cancer diagnosis, baseline values of alcohol intake (continuously) and use of HRT at blood sampling (yes/no). Body mass index and education were evaluated as potential confounders but were found unrelated to the endpoints of interest and therefore not included due to the relatively limited statistical power. The clinical parameters tumor grade at diagnosis (I, II, III, or non-ductal carcinoma), tumor size (mm) and tumor positive axillary lymph nodes (yes/no) were also evaluated, but as these variables could potentially be part of the biological pathway it was decided not to include them into the final model. Inclusion of the clinical parameters into the models despite this concern resulted in unchanged conclusions (results not shown). Women diagnosed with breast cancer within the first year after baseline were excluded to evaluate a potential effect of undiagnosed disease. This did, however, not influence the final results (results not shown) and this group was re-included.

Concentrations of AA-Hb, GA-Hb, the GA-Hb/AA-Hb ratio, as well as the two markers summed were included into the model as linear variables. The units (25 pmol/g globin) used for estimation in the linear analyses corresponds approximately to the inter quartile range of AA-Hb and GA-Hb concentrations. Before entering the Hb concentrations into the model, the linearity of the associations were evaluated by linear splines with three boundaries placed at the quartile cutoff points. None of the associations showed signs of deflection or threshold values. Values of GA-Hb below LOQ were entered into the models with their measured value but information of LOQ status was included into the model by use of an indicator variable.

Two-sided 95% confidence intervals (95% CI) for the hazard rates (HR) were calculated on the log HR scale. SAS (release 9.1) was used for statistical analyses.

3. Results

The 420 breast cancer cases were followed for a median of 10 years, during which 110 died and of those 80 had breast cancer as the primary cause of death.

Descriptive characteristics of the breast cancer cases at cohort baseline and breast cancer diagnosis are shown in Table 1. Compared to all breast cancer cases, the women that had died during follow-up seemed to be less frequent users of HRT and to have smoked for fewer years (among those ever smoked). Baseline concentrations of AA-Hb, GA-Hb, the two adducts summed or the ratio between them did not clearly differ between the women that died during follow-up and the entire group of breast cancer cases, but the levels of both AA-Hb and GA-Hb were noticeably higher among smokers than non-smokers.

Among non-smokers (Table 2), a higher AA-Hb concentration was associated with a slight tendency towards both higher breast cancer specific and higher all cause mortality. Adjustment for potential confounders tended to slightly strengthen the estimates, though the estimates still did not reach statistical significance. When considering the sub-group of women diagnosed with an estrogen receptor positive tumor, the estimates were further strengthened and reached statistical significance with regard to breast cancer specific mortality (HR (95% CI): 1.31 (1.02–1.69), per 25 pmol/g globin). Results are not shown for the subgroup of women diagnosed with an estrogen receptor negative tumor due to insufficient statistical strength.

Higher GA-Hb concentrations were found associated to higher breast cancer specific mortality after adjustment for the potential confounders, where a 25 pmol/g globin higher GA-Hb concentration was associated with a more than 60% higher risk of dying from breast cancer during the follow-up period (HR (95% CI): 1.63 (1.06–2.51)). When considering the sub-group of women diagnosed with an estrogen receptor positive tumor, the hazard of dying from breast cancer was doubled (HR (95% CI): 2.23 (1.38–3.61)) for a 25 pmol/g globin higher GA-Hb.

For all cause mortality, higher levels of GA-Hb were only statistically significantly associated to a higher hazard among women with an estrogen receptor positive tumor (HR (95% CI): 1.69 (1.06–2.69), but all the estimates were in the expected direction (above 1.00).

Also the GA-Hb/AA-Hb ratio and the summed concentrations of AA-Hb and GA-Hb were investigated according to breast cancer specific and all cause mortality (results not shown). These associations did not show any clear pattern and the only statistically significant finding was observed for the summed concentrations of AA-Hb and GA-Hb, where the risk factor adjusted estimate was associated with a higher breast cancer specific mortality among estrogen receptor positive cases (HR (95% CI): 1.24 (1.04–1.47) per 25 pmol/g globin).

Among breast cancer cases that were smokers at the time of blood sampling (Table 3), the picture was much less clear for AA-Hb with hazard rate estimates both below and above 1.00 (all statistically non-significant). For GA-Hb all estimates were above 1.00 and among women with an estrogen receptor positive tumor, GA-Hb was associated with a statistically significantly higher risk of dying from breast cancer (HR (95% CI): 1.72 (1.07–2.76) per 25 pmol/g globin). Neither for the GA-Hb/AA-Hb ratio nor the summed concentrations of AA-Hb and GA-Hb was any clear tendencies seen (results not shown).

4. Discussion

In this prospective cohort study evaluating the association between pre-diagnostic acrylamide exposure and mortality after breast cancer, higher rates of breast cancer specific mortality were seen among non-smoking breast cancer cases with higher concentrations of GA-Hb. The risk estimates seemed even stronger among those diagnosed with an estrogen sensitive breast tumor, where significant associations were seen for both breast cancer specific and all cause mortality. Similar tendencies were seen with regard to AA-Hb but only significantly for breast cancer specific mortality among estrogen receptor positive cases. For women smoking at the time of blood sampling, the only significant finding was for estrogen receptor positive cases, where higher GA-Hb was associated with higher breast cancer specific mortality.

That exposure to acrylamide is assessed by the medium term biomarkers AA-Hb and GA-Hb reflecting the internal exposure during the four months before blood sampling is the primary strength of the present study. AA-Hb and GA-Hb concentrations in blood seem to be relatively stable over time; within person correlation coefficients close to 0.80 have been shown for both adducts (median time between samples was 23 months) (Wilson et al., 2009). Other strengths are the prospective design, the relatively long and 100% complete follow-up for mortality and the ability to consider estrogen receptor status of the breast tumors. The major limitation concerns the limited statistical strength. Residual confounding from unmeasured dietary and lifestyle factors related to both acrylamide exposure and mortality after breast cancer is another potential problem; AA and GA forms adducts with Hb regardless of the route of exposure, foods (e.g., other substances formed through Maillard reactions), smoking or environmental. AA-Hb and GA-Hb adducts may therefore not only serve as a biomarker for AA and GA from different sources, but also as biomarkers for other toxic substances to which humans are exposed simultaneously with AA an GA.

To our knowledge, no previous studies have related AA-Hb and GA-Hb concentrations, or other measures of acrylamide exposure, to mortality after breast cancer diagnosis and it is consequently not possible to compare our results directly to previous studies. But looking into previous studies considering incidence of breast cancer and other endocrine related cancer sites and the hypothesis that acrylamide may influence the hormonal balance as presented in the recent review by Hogervorst et al. (2010) our findings could fit into an hypothesis of glycidamide worsening breast cancer survival through modification of the hormonal regulation. Hormone levels are among the most important factors associated with breast cancer prognosis (Folkerd and Dowsett, 2010) and all factors increasing the estrogenic effect may therefore be considered as relevant prognostic risk factors for breast cancer patients. That the association

Table 1
Descriptive baseline characteristics of all breast cancer cases and cases that died during follow-up.

	Breast cancer cases (n = 420)		Cases dead 31/12 2008 (n = 110)	
	Median (5–95%) or fraction		Median (5–95%) or fraction	
Age at cancer diagnosis	60	53–67	61	54–67
Alcohol intake (g/day)	11	1–44	11	1–55
Use of HRT at inclusion (%yes)	54		43	
Smoking				
Never (%)	40		38	
Former (%)	25		27	
Present (%)	34		35	
Smoking duration (years) – among ever smokers	28	0–44	22	0–46
Tobacco use (g/day) – among present smokers	15	3–25	15	5–25
Acrylamide (pmol/g globin)				
Non-smokers	35	19–99	36	20–83
Smokers	121	36–299	126	31–251
Glycidamide (pmol/g globin)				
Non-smokers	22	10–49	20	10–50
Smokers	58	17–130	62	12–152
GA-Hb/AA-Hb				
Non-smokers	0.59	0.24–1.04	0.56	0.19–1.04
Smokers	0.50	0.23–0.84	0.48	0.21–1.00
AA-Hb + GA-Hb (pmol/g globin)				
Non-smokers	57	30–137	57	34–123
Smokers	187	60–389	181	43–418

Table 2
Women not smoking at baseline. Hazard ratios (HR) and 95% confidence intervals (95% CI) for breast cancer specific mortality and all cause mortality according to hemoglobin adduct levels of acrylamide (AA-Hb) and glycidamide (GA-Hb) for all breast cancer cases and breast cancer cases diagnosed with an estrogen receptor positive tumor.

Non-smokers	All cases (n = 277)		ER+ cases (n = 197)[b]
	HR (95% CI) per 25 pmol/g globin	HR (95% CI)[a] per 25 pmol/g globin	HR (95% CI)[a] per 25 pmol/g globin
AA-Hb			
Breast cancer mortality (n = 55)	1.15 (0.93–1.41)	1.21 (0.98–1.50)	1.31 (1.02–1.69)
All cause mortality (n = 72)	1.09 (0.89–1.33)	1.14 (0.93–1.40)	1.22 (0.97–1.54)
GA-Hb			
Breast cancer mortality (n = 55)	1.37 (0.89–2.08)	1.63 (1.06–2.51)	2.23 (1.38–3.61)
All cause mortality (n = 72)	1.15 (0.75–1.76)	1.31 (0.85–2.03)	1.69 (1.06–2.69)

[a] Adjusted for time from blood draw to diagnosis, baseline levels of alcohol intake and use of hormone replacement therapy.
[b] Among estrogen receptor positive cases 44 died (31 from breast cancer).

in the present study seemed even stronger for those diagnosed with an estrogen receptor positive tumor is also in accordance with this hypothesis, as progression of this tumor type is known to be most strongly affected by endogenous estrogen levels (Folkerd and Dowsett, 2010). Glycidamide being an epoxide is highly reactive and very likely to react with amino and sulfhydryl groups within proteins possible altering their functionality. The greater reactivity of glycidamide compared to acrylamide may explain that the association is stronger and more consistent for GA-Hb compared to AA-Hb.

Although AA-Hb was only significantly associated with breast cancer specific mortality among women diagnosed with an estrogen receptor positive tumor in the present study, the tendencies were all in the same directions as seen for GA-Hb. Glycidamide is a metabolite of acrylamide and a correlation between AA-Hb and GA-Hb adduct levels is thus to be expected.

Whereas the estimates associating AA-Hb and GA-Hb to mortality (either breast cancer specific or all cause) all indicated higher mortality rates with higher concentrations among women that were non-smokers at blood sampling, the picture was much less

Table 3
Women smoking at baseline. Hazard ratios (HR) and 95% confidence intervals (95% CI) for breast cancer specific mortality and all cause mortality according to hemoglobin adduct levels of acrylamide (AA-Hb) and glycidamide (GA-Hb) for all breast cancer cases and breast cancer cases diagnosed with an estrogen receptor positive tumor.

Smokers	All cases (n = 143)		ER+ cases (n = 102)[b]
	HR (95% CI)per 25 pmol/g globin	HR (95% CI)[a] per 25 pmol/g globin	HR (95% CI)[a] per 25 pmol/g globin
AA-Hb			
Breast cancer mortality (n = 25)	1.02 (0.89–1.17)	0.95 (0.79–1.13)	1.10 (0.87–1.40)
All cause mortality (n = 38)	0.99 (0.88–1.11)	0.93 (0.80–1.09)	1.05 (0.88–1.26)
GA-Hb			
Breast cancer mortality (n = 25)	1.16 (0.91–1.49)	1.20 (0.86–1.68)	1.72 (1.07–2.76)
All cause mortality (n = 38)	1.06 (0.56–1.32)	1.09 (0.82–1.45)	1.39 (0.96–2.00)

[a] Adjusted for time from blood draw to diagnosis, baseline levels of alcohol intake, use of hormone replacement therapy, smoking duration and smoking intensity.
[b] Among estrogen receptor positive cases 22 died (12 from breast cancer).

clear for smokers. Tobacco smoking is an important source of acrylamide exposure and concentrations of AA-Hb and GA-Hb are known to be considerably higher in smokers than non-smokers (Vasper et al., 2008). Smoking is also a very important risk factor for cancer at many sites (IARC, 1986) as cigarette smoke contain several carcinogenetic polycyclic aromatic hydrocarbons of which benzopyrene is probably the most extensively studied (Hecht, 2002) and it is therefore especially important to carefully consider smoking status when acrylamide exposure is related to risk of cancer. Breast cancer is, however, one of the few cancer sites where smoking is not considered a strong risk factor (Terry and Rohan, 2002). Though the general carcinogenic effects of smoking probably affects tumor initiation in the breast (Terry and Rohan, 2002), smoking also has been ascribed anti-estrogenic effects (Kendall et al., 2007). It is therefore possible that the measured AA-Hb and GA-Hb concentrations in the present study are markers of both the potential of glycidamide to modify estrogenic effects and the potential anti-estrogenic effects of tobacco smoking. Estimates both below and above 1.00 with wide confidence limits are consequently not surprising findings. Still, in the sub-group where the finding was strongest among the non-smoking women (GA-Hb and breast cancer specific mortality among women diagnosed with an estrogen receptor positive tumor) a higher hazard was also evident among the smoking women.

An important aspect to consider regarding the current study is that the biomarkers (AA-Hb and GA-Hb) are measures of acrylamide exposure prior to breast cancer diagnosis. The study is, consequently, not able to determine if survival among women already diagnosed with breast cancer is affected by exposure to acrylamide. AA-Hb and GA-Hb are known to reflect acrylamide exposure within the approximately 4 months life-time of an erythrocyte (Bergmark, 1997; Paulsson et al., 2003) and in the present study the erythrocytes where sampled between 0 and 7 years before breast cancer diagnosis and death (or end of follow-up) occurred after a median of additionally 10 years.

Further studies are needed to consider the time aspect of acrylamide exposure with regard to mortality after breast cancer.

In vitro AA has been shown to increase methemoglobin formation, superoxide dismutase (SOD) activity, malondialdehyde (MDA) formation, hemolysis and decrease glutathione peroxidase (GSH-Px) activity, catalase (CAT) activity and reduced glutathione (GSH) levels in erythrocytes at exposure levels from 0.1 to 1 mM (Catalgol et al., 2009). However the relevance of these observations for humans is uncertain since a human exposure level of 20 μg/kg bw would give a maximum blood concentration of approximately 29 nM (Kopp and Dekant, 2009), a factor of more than 3400 lower than the lowest dose in the study by Catalgol et al. (2009). In addition 20 μg/kg bw is five times greater than the dietary acrylamide intake of 4 μg/kg bw per day among high consumers as estimated by (JECFA, 2005).

The same 420 breast cancer cases that form basis for the present study have previously been included in a study evaluating the associations between AA-Hb and GA-Hb concentrations and incidence of breast cancer (Olesen et al., 2008). Our previous study indicated an association between acrylamide exposure and incidence of primarily estrogen receptor positive breast cancer, though the findings seemed less clear than what is observed in the present study. Especially that the increased breast cancer incidence was strongest among smokers was of concern and is in contrast to our findings regarding breast cancer mortality, showing the most consistent effect among non-smokers, which is more biologically reasonable. A reason for the discrepancy between the two studies may be that it was not possible to stratify completely on smoking in the incidence study due to matching of cases and controls on other factors but not smoking. Only case–control pairs that were concordant (that is both case and control were accidentally either smokers or non-smokers) therefore contributed to the smoking-stratified estimates. In the present study, it was possible to stratify on smoking at blood sampling.

Though the findings of the present study seem to be in accordance with what was expected, it is important to consider that the hypothesis of acrylamide having endocrine related effects is only suggestive. Additionally, as this is an epidemiological study, potential impact of unmeasured factors can neither be excluded nor quantified and the findings must be interpreted taking this into account. Further research, both experimentally and epidemiologically, is therefore needed before any implications for public health can be properly assessed.

In conclusion, we found that higher pre-diagnostic concentrations of GA-Hb, a marker of internal glycidamide exposure, was associated to higher risk of dying from breast cancer among non-smoking breast cancer patients, especially among those diagnosed with an estrogen receptor positive tumor. Future studies are needed to confirm our findings.

Conflicts of interest statement

None of the authors have any conflicts of interest regarding the present manuscript.

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