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Estrogen Metabolism and Familial Risk of Breast Cancer

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It has been suggested that women who metabolize a larger proportion of their natural estrogen via the 16α-hydroxy pathway may be at significantly elevated risk of breast cancer compared to women who metabolize proportionally more estrogen via the 2-hydroxy pathway. This study evaluates whether the ratios of 16α-OHE1 to 2-OHE1 are higher in urine of premenopausal women at "high" than at "low" familiar risk of breast cancer; and whether the ratio is elevated in cases independent of total urinary estrone (E1), estradiol (E2) and estriol (E3). Early morning urine samples are collected from 100 premenopausal women at "high" and 100 premenopausal women at "low" risk of breast cancer. All subjects are sisters or daughters of subjects participating in one of three case-control studies of breast cancer at our institution. Five estrogen metabolites in urine are determined: 16α-OHE1, OHE1, E1, E2 and E3 conjugates. The data collection is in progress.

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(5) Introduction:

16α- and 2-hydroxylation of E1

The extent to which E1 is metabolized via the 16α-hydroxylation pathway may be associated with breast cancer risk (1-3). The two main pathways for metabolizing E1 are via 16α-hydroxylation and 2-hydroxylation. The 16α-metabolites, are biologically active; the 2-metabolites are not (4-6).

Women at high risk of breast cancer

Women who have a first degree relative who has had breast cancer are themselves at increased risk. The risk is higher if the relative had bilateral compared to unilateral breast cancer, or if the diagnosis was made at an early age. Women who had a first degree relative diagnosed with bilateral breast cancer before age 50 may have as much as a five-fold elevated risk (7-12).

The increased risk in first degree family members may be due to shared environmental factors and/or genetic factors that increase the susceptibility to breast cancer. One possible mechanism for the increased risk (or part of the increased risk) could be the pathway by which estrogen is metabolized (13).

The hypothesis to be tested is that women at 'high-risk' of breast cancer metabolize a significantly higher amount of E1 through 16α- than 2-hydroxylation compared to 'normal-risk' women, independent of total urinary E1, E2 and E3. We expect the ratios of 16α-hydroxy-metabolites to 2-hydroxy-metabolites to be statistically significantly higher in 'high-risk' than in 'normal-risk' women.

(6) Body

METHODS

Case selection: We are in the process of identifying 100 women at high risk of breast cancer. Premenopausal sisters and daughters of patients with premenopausal uni- or bilateral breast cancer who participated in a genetic-epidemiologic study (14) or a case-control study of breast cancer (15-16), and who have never themselves been diagnosed with breast cancer, represent the 'cases' in this study.

Case selection- eligibility criteria:

1. Premenopausal sister or daughter of a woman with either
   a) premenopausal bilateral breast cancer who was identified through the Los Angeles County Cancer Surveillance Program (LACCSP; and NCI SEER registry), and participated in a genetic-epidemiologic study of breast cancer (P.I. Robert W. Haile), or
   b) unilateral breast cancer diagnosed before the age of 40 who was identified through the LACCSP, and participated in a study of breast cancer (P.I. Leslie Bernstein)
2. Age between 20 and 50 years old.
3. Not currently pregnant or breast feeding.
4. Never been diagnosed with cancer.
5. Over the past 6 months: not used medications that may interfere with estrogen metabolism (estrogen, progesterone, oral contraceptives, tamoxifen, cimetidine, thyroxin, or omega-3 fatty acid supplements).
6. Over the past 3 months: not had general anesthesia.
7. Living in California.

Control selection- eligibility criteria

We originally proposed to use 100 age-matched female friends of the cases as controls. However, because of concerns regarding use of friends as controls, as well as substantial problems obtaining permission from the cases to contact their friends (less than 10% of cases were willing to provide us with names of friends), we had to find other controls. We are now using daughters or sisters of controls who participated in one of two studies of breast cancer conducted by Dr. Leslie Bernstein in our department.

Eligible controls are:
1. Premenopausal sister or daughter of a woman who participated in either
   a) study of breast cancer under the age of 40 (P.I. Leslie Bernstein) or
   b) the USC part of the Women’s CARE study (USC P.I. Leslie Bernstein)
2. Age between 20 and 50 years old.
3. Not currently pregnant or breast feeding.
4. Never been diagnosed with cancer.
5. Over the past 6 months: not used medications that may interfere with estrogen metabolism (estrogen, progesterone, oral contraceptives, tamoxifen, cimetidine, thyroxin, or omega-3 fatty acid supplements).
6. Over the past 3 months: not had general anesthesia.
7. Living in California.

If more than one member is eligible in one family, then the youngest member is enrolled in our study.

Data acquisition

We contact women who have participated in one of our previous studies, and ask them for permission to contact any daughters or sisters they may have living in California, and who are between the ages of 20 and 50 years old. We contact these daughters/sisters, and find out if they are eligible for the study.

We obtain urine samples from the women during the follicular phase, i.e. during the first 10 days of the menstrual cycle. Once a woman is found to be eligible, she is asked to call us when the next menstrual cycle starts.

A box containing a 100 ml urine vial with a 100 mg ascorbate tablet, a small cooler with an ice pack, an informed consent form, and a questionnaire on recent intake of medication, alcohol and specific foods, are shipped to each eligible woman who agrees to participate. The participants are asked to place the urine sample in the cooler with the ice pack (previously frozen by the participant) immediately after it has been produced, and to enclose a signed informed consent form and the completed questionnaire on alcohol intake and current medication. The cooler is shipped to us by overnight express mail. The urine samples are divided into four samples of approximately 12 ml and immediately frozen at -70°C until shipped to the processing laboratories. Dr. Bradlow at the Strang-Cornell Cancer Research Laboratory will perform the 16α-OHE1 and 2-OHE1 assays while Dr. Stanczyk at Los Angeles County/University of Southern California (LAC/USC) Women's Hospital will perform the E1, E2 and E3 assays. The only identifiers on the samples are code numbers ensuring that the laboratories will be blinded as to case or control status of the individual samples. Dietary questionnaires are sent to each woman.
one week after she provides the urine sample. The dietary questionnaire is returned by mail in a stamped envelope we provide.

RESULTS

We have so far contacted 461 women with premenopausal uni- or bilateral breast cancer and 413 controls who participated in one of our previous studies (see above). Initial attempts were made to contact these women by telephone. However, most of these women were last contacted as part of the previous study some time before 1990. Therefore, a large number of the telephone numbers were no longer current. We therefore early decided to make the initial contact by mail, with a request for a forwarding address. We have sent up to three letters to some of these women. We have attempted to tract women whose current address is unknown (their letters have been returned with no forwarding address), through the records of the California Department of Motor Vehicles. In addition, we have attempted to call non-responders, using their old number, or any new number we have found using the names inverse street directory. Completing this search and obtaining responses from these women turned out to be a much greater task than expected (first part of task 1 in original Statement of Work (SOW)).

Of the responses obtained so far from the cases and controls in one of our previous studies, 181 cases and 124 controls had at least one daughter or sister between the ages of 20 and 50 living in California (total of 262 case daughters or sisters and 205 control daughters or sisters). We have contacted all of these case and control daughters and sisters. When there is more than one daughter/sister in each family, we include the youngest one above age 20 if eligible. This means that we must await a response from the younger potential eligible member before we decide who should be included in the study. To date we have obtained responses from approximately 180 case daughters or sisters and 140 control daughters or sisters. Since only one member from each family is eligible, this means that we have a total of 78 eligible case daughters or sisters and 28 eligible control daughters or sisters. Major reasons for ineligibility include current oral contraceptive use (30%), current smokers (10%), other medications (5-10%), irregular periods (10% of controls), currently pregnant/breast feeding (10% of controls). Finding eligible women (especially controls) therefore represented an enormous problem (task 1 in SOW). We have so far collected urine samples and dietary questionnaires on 70 case daughters or sisters and 20 control daughters or sisters (tasks 2a and 2c in SOW). We are contacting eligible women on a regular basis to remind them of contacting us when their menstrual period begins. We are now making a final effort to increase the number of control participants.

We have not started shipping urine samples to the laboratories (tasks 2b and 3 in SOW) for two reasons: 1) due to the substantial problems finding eligible controls, and 2) because our study coincided with a reproducibility/validity study of the EIA assays of 16α- and 2-OHE1 conducted by Dr. Regina Ziegler, NCI. The results of this validation study indicated that the assays must be adjusted (Regina Ziegler, personal communication), and as a result the new adjusted assays must again be validated. We therefore requested (and obtained) a 1-year no-cost extension of this grant.

All dietary questionnaires will be shipped to Harvard once the study is completed. The other risk factor information obtained for this study is being prepared for key-punching. The data will be key-punched all at one time when the data collection is completed.
(7) Conclusions

We have no laboratory results we can draw implications from at this point. It is also too early to suggest changes for future projects, except perhaps that it would be useful to request funds for a separate validity/reproducibility study whenever a new method is being used.

(8) References