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Association between Epstein–Barr virus infection and risk for development of pregnancy-associated breast cancer: Joint effect with vitamin D?

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ABSTRACT

Background: Few studies have evaluated the role of the ubiquitous Epstein–Barr virus (EBV) infection, together with levels of the immunomodulator, vitamin D, in different breast cancer entities. We studied, prospectively, the association of EBV and vitamin D status with the risk of pregnancy-associated breast cancer (PABC), breast cancer diagnosed during pregnancy or 1 year post-partum, using a nested case–control study.

Methods: Serum vitamin D and antibodies to EBV were measured for 108 PABC cases of the Finnish Maternity Cohort, and 208 controls matched for date of birth, date of sampling and parity. The joint effect of vitamin D and EBV on the risk of PABC was evaluated.

Results: EBV seropositivity was generally not associated with the risk of PABC. Among individuals with sufficient ($\geqslant$75 nmol/l) levels of vitamin D, we, however, found similar increased risk estimates for PABC associated with serum immunoglobulin G (IgG) antibodies to EBV early antigens [odds ratio (OR) = 7.7, 95% (confidence interval) CI 1.4–42.3] and the viral reactivator protein, ZEBRA (OR = 7.8, 95% CI 1.1–61.2).

Conclusion: Immunological markers of EBV reactivation status among individuals with sufficient vitamin D levels were consistently associated with increased risk of the disease. This suggests that EBV reactivation may be an indicator of the progression of breast cancer occurring soon after pregnancy, while the virus probably is not the aetiological agent.

1. Introduction

Established risk factors of breast cancer explain only about 50% of the disease aetiology.[1] This has prompted interest on environmental agents, including viral infections in breast carcinogenesis. Epstein–Barr virus (EBV), noted as a class 1 carcinogen over a decade ago by the International Agency for Research on Cancer (IARC),[2] has been of particular interest.[3–5]

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The hypothesis that EBV is involved in the aetiology of breast cancer was originally based on evidence that mothers with latent EBV infection shed the virus in their breast milk.[6] On the other hand, lymphoepithelioma-like carcinomas such as some gastric cancers have long been known to be positive for EBV,[7] and known breast cancer subtypes portray comparable features with lymphocytic infiltration. EBV DNA has been identified in benign breast tissue samples of both immunosuppressed and immunocompetent individuals[8] and EBV-positive lymphoma growth has been described also in the breast tumours.[9]

The detection rate of EBV in breast carcinoma cells varies. Some studies report the absence of the EBV genome,[10–12] while others report detection rates between 20% and 60%.[13–15] The disparate findings have been attributed to methodological variation.[16] Serological studies have evaluated the association between EBV antibodies and the risk of breast cancer, albeit also with controversial results.[3–5] These findings suggest that the role of EBV in a sub-group of breast cancer aetiology may be either causal or that of a bystander in conjunction with EBV reactivation.

Pregnancy-associated breast cancer (PABC) is diagnosed most often during pregnancy or 1 year post-partum. The incidence of PABC is low (1–3 in 10,000 pregnancies), but has been increasing,[17] as women elect to delay childbearing to their third and fourth decades. PABC not infrequently has lymphoepithelial vascular invasion,[18,19] probably involving also EBV-positive cells. We evaluated the association between pre-existing EBV infection and the risk of PABC.

EBV reactivation takes place not infrequently during pregnancy.[20,21] Following EBV reactivation, up-regulation of viral oncogenes and cellular growth regulating genes, such as the latent membrane protein (LMP1), takes place. Also, vitamin D status modulates the immune response to and reactivation of EBV, and interplay of the two has recently been suggested to play a role in chronic disease (multiple sclerosis) development,[22,23] often following pregnancy. It is possible that EBV reactivation, by vitamin D levels, is associated with risk of breast cancer.

In a previous study, we showed that higher vitamin D levels are associated with an increased risk of PABC.[24] This study further evaluates the association between serum antibodies indicating EBV infection or reactivation and the risk of subsequent development of PABC in a case–control study nested within the Finnish Maternity Cohort (FMC) involving females with adequate or inadequate supply of vitamin D.

2. Materials and methods

2.1. Study population

The Finnish Maternity Cohort (FMC) is a serum repository of over 1.6 million pregnant women serum samples established in 1983 in Finland, with a national coverage among pregnant women of approximately 98%. The samples are withdrawn at municipal maternity care units during the first trimester of pregnancy (gestational weeks 10–12), following an informed consent, for screening of congenital infections. After the screening, 1–3 mL volume of serum is stored at –25 °C in polypropylene cryo vials at the National Institute for Health and Welfare, Oulu, Finland.[25]

Cases and controls for this study were derived from a linkage of the FMC and the Finnish Cancer Registry (FCR), the national population-based cancer register in Finland. The FCR established in 1953 has a national coverage which is virtually complete with no loss to follow-up.[26] The record-linkage was done at the FCR using unique personal identity codes given to all residents in Finland.

Our study material constituted 111 incidence density sampled PABC cases–control pairs that were previously used.[24] Three case samples were excluded because of insufficient sample volume. Each case was matched to 2 controls for date of birth (±1 year), time of serum sampling (±14 days) and parity (±1). Parity was defined as the number of pregnancies at the time of sample withdrawal. A total of 208 controls were available for analysis. Data for serum 25-OHD for all samples were available from our previous analysis,[24] and were used to determine joint effects of vitamin D and EBV past infection. The study was approved by the ethical committee of the National Institute for Health and Welfare, Finland.

2.2. Laboratory analyses

Maternal immunoglobulin G (IgG) antibodies to EBV nuclear antigen (EBNA) and early antigen (EA) were assessed by commercial enzyme-linked immunosorbent assays (ELISAs) based on recombinant proteins (Biotest AG, Frankfurt, Germany). Cut-off values, defining positive or negative samples on all plates, were calculated by following the manufacturer's recommendations. Samples that were EBNA negative were also assessed for VCA IgG to evaluate past infection. In addition, EBV Zebra IgG antibodies were evaluated by an in-house-made peptide-based ELISA, as previously described.[27,28] Laboratory analyses were performed on coded samples with case–control status masked.

2.3. Statistical analysis

Descriptive statistics were calculated for cases and controls. Relative risks (RR) expressed as odds ratio (ORs) with 95% confidence intervals (CI) were estimated by conditional logistic regression for the matched case–control triplets using SPSS 15 for windows (SPSS Inc., Chicago, IL). After categorising by sufficient levels of vitamin D (75 nmol/L of serum 25-OHD was used as the cut-off value[29]), the matching criteria were digressed. Thus, unconditional logistic regression was used to calculate the risk estimates for the serological EBV markers and PABC risk, adjusting for the matching criteria. A two-sided p value <0.05 was considered statistically significant.

3. Results

Mean age at serum sampling for the PABC cases was more than that of the pregnant women in Finland (34.4 years versus 31.5 years (Table 1)). Almost all the samples were positive for EBNA IgG antibodies (95.4% and 96.3% for cases and controls, respectively). Seventy-three percent and 12% of the cases were positive for EBV EA and ZEBRA IgG antibodies,

Table 1 – Baseline characteristics of pregnancy-associated breast cancer cases and controls samples of the Finnish Maternity Cohort (FMC).

	Cases	Controls
Number (*N*)	108	208
Mean age[a]	34.2	34.4
Gestational day (d)	77.1	77.9
Parity	2.0	2.3
Follow-up time[a]		1
Age at diagnosis (range)	35.6 (24.9–43.9)	

[a] Calculated in years

Table 2 – Relative risk odds ratios (OR) with 95% confidence intervals (CI) for pregnancy-associated breast cancer in relation to the detection of positive immunoglobulin G (IgG) antibodies to Epstein–Barr virus (EBV) antigens in the Finnish Maternity Cohort.

Seropositivity	Number of			
	Cases	Controls	ORs	95% CI
EBNA				
No	6	8	1	
Yes	102	200	0.7	0.2–2.2
EA				
No	30	73	1	
Yes	78	135	1.5	0.9–2.5
ZEBRA				
No	94	191	1	
Yes	14	17	1.4	0.7-2.8

respectively, compared to 64% and 8.9% of the controls (Table 2). Seventy-seven percent (76.9%) of the cases were positive for serological markers of EBV reactivation (anti-EA or anti-ZEBRA) compared to 66.8% for controls.

The concentrations of anti-EA IgG and anti-ZEBRA IgG were significantly correlated ($p < 0.04$). There was no association between the risk of PABC and EBV antibodies to the presence of EBNA, EA and ZEBRA antigens (Table 2). Individuals positive for either anti-EA IgG or anti-ZEBRA IgG antibodies had a somewhat increased risk of PABC with borderline statistical significance (OR = 1.7, 95% CI 1.0–2.8).

Among individuals with sufficient levels of serum 25-OHD ($\geqslant$75 nmol/l), we noted a significantly increased risk of PABC associated with positivity for both serum EA IgG antibodies (OR = 7.7, 95% CI 1.4–42.3) and ZEBRA IgG antibodies (OR = 7.8, 95% CI 1.1–61.2) (Table 3). To increase the sensitivity for the determination of EBV reactivation, we considered positivity for either EA or ZEBRA IgG antibodies. The presence of either EA or ZEBRA IgG antibodies was significantly associated with the risk of PABC (OR = 12.1 95% CI 1.3–107.3). No such observations were made among individuals with less than the sufficient levels of vitamin D (Table 3).

4. Discussions

In general, we found no association between pre-existing EBV infection and the risk of development for pregnancy-associated breast cancer. Positivity for immunological markers of EBV reactivation among individuals with sufficient levels of vitamin D was, however, associated with a significantly increased risk of breast cancer occurring during or soon after pregnancy.

Over 96% of the controls were EBV seropositive, comparable to previous data from pregnant women populations in Finland[30] and the United States of America.[20] The finding that PABC cases were older at serum withdrawal compared to other women in our cohort is consistent with the increasing

Table 3 – Odds ratios(OR) with 95% confidence intervals (CI) for pregnancy-associated breast cancer and EBV EBNA, EA and ZEBRA IgG antibody positivity among women with sufficient and less than sufficient levels of vitamin D in first trimester serum samples of the Finnish Maternity Cohort.

Seropositivity		Number of			
		Cases	Controls	OR[a]	95% CI
Vitamin D (<75 nmol/l)					
EBNA	No	6	8	1	
EBNA	Yes	89	176	0.9	0.2–3.5
EA	No	28	60	1	
EA	Yes	68	124	0.8	0.4–1.6
ZEBRA	No	85	169	1	
ZEBRA	Yes	10	15	1.5	0.5–4.9
Vitamin D ($\geqslant$75 nmol/l)					
EBNA	No	0	0		
EBNA	Yes	13	24	n.a.	
EA	No	2	14	1	
EA	Yes	11	10	7.7	1.4 – 42.3
ZEBRA	No	9	22	1	
ZEBRA	Yes	4	2	7.8	1.1 – 61.2

Vitamin D levels defined as insufficient (serum 25-OHD <75 nmol/l) and sufficient (serum 25-OHD $\geqslant$75 nmol/l).
n.a = not available.
[a] Adjusted for age and season of sampling.

incidence of PABC in women postponing childbearing to later ages. EBV reactivation, however, is thought to be independent of the maternal age at pregnancy.[20]

The null association between past EBV infection and the risk for subsequent development of PABC in this study is consistent with a previous study on EBV and premenopausal breast cancer.[3] In that cross-sectional study, no association was found between positivity for EBV and the risk of breast cancer in young women. In another study,[4] the authors observed that the mean anti-EBNA IgG antibody levels were significantly higher in breast cancer cases than in controls with benign breast disease. In a recent case–control study nested within the Janus Serum Bank cohort,[5] EBV IgG antibody levels in serum samples drawn at least 4 years before breast cancer diagnosis were not associated with the risk of the disease.

This is the first study, to our knowledge, to evaluate the interplay between prediagnostic EBV reactivation and vitamin D with the risk of PABC. Only 12% of our cases and controls had sufficient levels of vitamin D, as vitamin D insufficiency is very common in pregnant women.[31,32] Positivity for both serum anti-EBV ZEBRA and anti-EBV EA IgG antibodies indicated the occurrence of EBV reactivation, almost exclusively in individuals with sufficient levels of vitamin D. These women, who were positive for anti-EBV EA or ZEBRA IgG antibodies, had a significantly increased risk for PABC.

The active form of vitamin D, dihydroxyvitamin D_3, is a potent immunomodulator and may modulate the immune response to EBV infection by suppressing T-cell proliferation.[22,23] It is possible that higher levels of vitamin D reduce the immune surveillance of EBV, thus promulgating viral reactivation from EBV harbouring B cells. It is equally possible that EBV reactivation was caused by the true cause of PABC and just became detectable in individuals with sufficient vitamin D status.

The discrepant results on the association between breast cancer and EBV infection in previous studies, which are based on EBV serological markers, are probably due to the differences in the serological markers of EBV infection used and the heterogeneity of the breast cancer cases. Methodological variation in detecting EBV has also been suggested to explain the results.[16] In this study, we employed four serological markers of EBV infection and found that the expression of EBV early antigens consistently suggested the reactivation of EBV in a proportion of PABC cases. The longitudinal and population-based nature of sample collection and cancer registration minimises the possibilities that our results are due to reverse causality bias or chance introduced because of case misclassification. Finally, it is possible that breast cancer heterogeneity may have diluted the association between past EBV infection and the risk of PABC, particularly because sub-groups were not categorised by hormone receptor status. The small sample size of this study, in particular, warrants further studies.

In conclusion, we found no association between past EBV infection and the subsequent risk of PABC. However, EBV reactivation among individuals with sufficient vitamin D levels was associated with increased risk of the disease. EBV reactivation may be an indicator of the progression (not an aetiological agent) of breast cancer occurring during pregnancy.

Conflict of interest statement

None declared.

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