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Does the evidence for an inverse relationship between serum vitamin D status and breast cancer risk satisfy the Hill criteria?

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A wide range of epidemiologic and laboratory studies combined provide compelling evidence of a protective role of vitamin D on risk of breast cancer. This review evaluates the scientific evidence for such a role in the context of the A.B. Hill criteria for causality, in order to assess the presence of a causal, inverse relationship, between vitamin D status and breast cancer risk. After evaluation of this evidence in the context of Hill's criteria, it was found that the criteria for a causal relationship were largely satisfied. Studies in human populations and the laboratory have consistently demonstrated that vitamin D plays an important role in the prevention of breast cancer. Vitamin D supplementation is an urgently needed, low cost, effective, and safe intervention strategy for breast cancer prevention that should be implemented without delay. In the meantime, randomized controlled trials of high doses of vitamin D_3 for prevention of breast cancer should be undertaken to provide the necessary evidence to guide national health policy.

Prevention of breast cancer is one of the greatest challenges currently facing public health researchers and policymakers. Globally, a wide range of epidemiologic studies have shown an inverse relationship between sunlight or ultraviolet-B (UVB) irradiance (the main source of circulating vitamin D in humans),[1-8] oral vitamin D intake,[9-14] and serum 25-hydroxyvitamin D [25(OH)D] concentration (the main circulating vitamin D metabolite),[15-22] with risk of breast cancer.

There is also substantial laboratory evidence that vitamin D metabolites exert several powerful anti-carcinogenic effects on breast cancer cells including: induction of apoptosis,[23] inhibition of angiogenesis,[23] and helping to maintain breast epithelial cells in a well-differentiated state via upregulation of the glycoprotein e-cadherin.[24]

In order to assess the presence of a causal, inverse relationship, between vitamin D status and breast cancer risk, this review evaluates the scientific evidence and frames it in the context of Hill's criteria.[25] In epidemiology, the seven most important criteria postulated by Hill are used to determine whether or not a causal relationship exists between a given exposure and disease.[26] Briefly, the Hill criteria are as follows:

(1) Presence of a temporal relationship. The exposure must precede the disease.

(2) Strength of the association. This is the magnitude of the relationship between the exposure and disease, usually expressed by the relative risk or odds ratio in epidemiological studies.

(3) Presence of a dose-response relationship. Increasing or decreasing exposure to a given factor results in a corresponding increase or decrease in risk of the disease.

(4) Consistency. The results of studies investigating the relationship between a given exposure and disease are consistent across most or all studies.

(5) Biological plausibility. The relationship between a given exposure and disease fits with current scientific knowledge of the biological mechanisms of that disease.

(6) Consideration of alternative hypotheses. Alternative hypotheses regarding the cause of a given disease must be considered and ruled out before inferring a causal relationship between the disease and the exposure of interest.

(7) Experiment. The disease can be prevented or treated by administration of the appropriate agent or lack thereof.

Temporal Relationship

The first criterion in establishing causality is the presence of a temporal relationship. In other words, if a given exposure is thought to cause a disease, then the exposure must precede the onset of disease. In studies of serum 25(OH)D and breast cancer, this criterion is satisfied. For example, in the randomized controlled trial performed by Lappe and colleagues,[14] 1,179 cancer-free women receiving 1,100 IU/day of vitamin D_3 experienced a 77% lower risk from all cancers (including breast cancer) over a three year period (years 1–4). Overall, there were 9 cases of cancer in the vitamin D group compared with 15 cases in the placebo group.

In a meta-analysis of 11 case-control and nested case control studies by Mohr et al.,[27] there was a 13% lower risk of breast

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cancer in women in the highest quintile vs. the lowest quintile of serum 25(OH)D, combining all studies that used pre-diagnostic sera to measure vitamin D status (p = 0.04). In all of the nested case-control studies, serum 25(OH)D was measured before case diagnosis, with mean time between serum draw and case diagnosis ranging from 3–7 y. Therefore, serum 25(OH)D measurements may not have been representative of 25(OH)D levels during the relevant window of time in vitamin D is most active against the development of a tumor, which appears to be maximal at 3 mo preceding diagnosis.[28] Although the Rejnmark study was not considered a nested case-control study in the Mohr et al. meta-analysis, blood samples for vitamin D measurement were obtained slightly before diagnosis of breast cancer via mammography and biopsy.[21] In the Rejnmark study, women with serum 25(OH)D concentration greater than 34 ng/ml had a 48% lower estimated risk compared with women with less than 24 ng/ml.

In contrast, the effect of serum 25(OH)D concentration on risk was much stronger in ordinary case-control studies where serum 25(OH)D levels were measured during or shortly after diagnosis. While an alternative explanation for the strong inverse association observed in these studies is that the breast neoplasm may be responsible for lower serum 25(OH)D levels, this is highly unlikely and there is no biological basis or evidence to support it.

In the study performed by Abbas et al., there was a 50% lower risk of breast cancer in women in the highest quartile of serum 25(OH)D compared with the lowest (p = 0.001). This result did not change when cases whose 25(OH)D concentration was measured longer than six months after diagnosis were excluded.[17]

Strength of Association

A strong relationship between exposure and disease is necessary to satisfy this criterion. The inverse association between serum 25(OH)D and risk of breast cancer ranged from an odds ratio of 0.20 (95% confidence interval 0.1–0.5),[20] corresponding to an 80% reduction in risk for the highest vs. lowest quantile of 25(OH)D concentration, to a non-statistically significant odds ratio of 1.20 (95% CI 0.9–1.6).[29] However, in the meta-analysis performed by Mohr et al., there was an overall 47% lower risk (pooled odds ratio 0.63, $p < 0.0001$) for all studies combined, including ordinary and nested case-control designs.[27] When the analysis was restricted to ordinary case-control studies, there was a lower risk of breast cancer when comparing subjects in the highest vs lowest quantile of 25(OH)D concentration (pooled odds ratio 0.41, $p < 0.0001$). In addition, there was a 13% reduction in risk (pooled odds ratio 0.87, $p < 0.04$) when only nested case-control studies were included in the pooled analysis. This is important because the effect was still present in these studies even though serum 25(OH)D levels were often measured years before case diagnosis and may not have been an accurate representation of 25(OH)D levels during the relevant period of time for maximal action of vitamin D on risk of breast cancer, resulting in non-differential misclassification of exposure, which would increase the tendency to observe a null finding.[30]

The inverse relationship between serum 25(OH)D concentration and breast cancer risk in the meta analysis is of a sufficiently high magnitude,[27]as described above, to satisfy this criterion.

Presence of a Dose-Response Relationship

Seven of the 11 published case-control studies of 25(OH)D levels and breast cancer risk demonstrate a dose-response gradient with higher levels of serum 25(OH)D resulting in a nonlinear decrease in risk.[15-21] This is similar to the inverse, dose-response relationship observed between serum 25(OH)D levels and risk of colorectal cancer.[31] In the meta-analysis by Mohr et al.,[27] the dose-response relationship was estimated using data from 11 case-control and nested case-control studies. A downward, nonlinear trend, was observed with higher concentrations of 25(OH)D ($p < 0.001$) in both types of studies.

Consistency

The role of vitamin D in prevention of breast cancer is strongly supported by six important lines of evidence. These lines of evidence all intersect at the conclusion that vitamin D and its metabolites play a paramount role in the prevention of breast cancer:

(1) Four studies that found a positive association between latitude or an inverse association between UVB irradiance and breast cancer incidence or mortality.[1-3,32]

(2) Five ordinary case-control studies that found an inverse association between serum 25(OH)D and breast cancer risk.[17-21]

(3) Two nested case-control studies that found an inverse association between serum 25(OH)D and breast cancer risk,[15,16] although there were five that individually did not detect a statistically significant association.[29,33-36] However, a long lag time between serum 25(OH)D measurement and case diagnosis in nested case-control studies is the most likely explanation for lack a of statistically significant relationship between serum 25(OH)D levels and breast cancer risk.[28]

(4) Substantial evidence from laboratory studies.[23]

(5) Six studies of oral intake of vitamin D that found an inverse association with risk of breast cancer in humans,[9-13,37] although there were four that individually did not detect a statistically significant association.[38-41]

(6) A randomized controlled trial that identified a 77% reduction in overall incidence of all invasive cancers in postmenopausal women, including a non-significant reduction in incidence of breast cancer.[14]

Furthermore, due to increased pigmentation, African-American women have lower serum 25(OH)D concentrations than Caucasian women.[42] Therefore, higher risk of breast cancer has been consistently observed in African-American women compared with Caucasian women.[43-45]

Biological Plausibility

In addition to the abundant evidence from observational studies, the powerful anti-carcinogenic properties of vitamin D metabolites,

especially $1,25(OH)_2D$, have been demonstrated in numerous laboratory studies. Studies have shown that $1,25(OH)_2D$ helps to maintain breast epithelial cells in a well differentiated state and downregulates expression of aromatase through several mechanisms such as inhibiting production of the COX-2 enzyme.[46] Expression of aromatase also is required for synthesis of estrogen and may therefore play a role in the prevention by vitamin D of estrogen receptor (ER) positive breast cancers.[46]

In human breast cancer cell cultures, $1,25(OH)_2D$ has been shown to induce apoptosis and inhibit factors that stimulate cell proliferation.[23] It has also been shown that $1,25(OH)_2D$ can inhibit angiogenesis in endothelial cell cultures in response to pro-angiogenic factors such as the signal protein Vascular Endothelial Growth Factor (VEGF).[47] Furthermore, COX-2 has also been shown to increase angiogenesis, so by downregulating expression of COX-2, $1,25(OH)_2D$ further blocks angiogenesis.[23,46]

Several other mechanisms have been proposed for the prevention of human breast cancer through achieving vitamin D sufficiency. One of the main attributes of malignancy in breast cancer is the loss of adhesion between cells in the terminal ductal epithelium of the breast.[48] This loss of adhesion can be partly attributed to the downregulation of e-cadherin that occurs in vitamin D deficiency.[49] E-cadherin is a glycoprotein that serves as a sort of glue that helps to keep cells in close contact, and, as a result, in a well-differentiated state. Breast cancer prognosis is significantly worse in the total absence of e-cadherin expression due to loss of differentiation and an increase in metastatis.[24]

Under the vitamin D-cancer prevention hypothesis, breast cancer occurs in several distinct phases that can be explained by a theoretical model termed the Disjunction-Initiation-Natural selection-Overgrowth-Metastasis-Involution-Transition (DINOMIT) model.[50] In the first phase of the DINOMIT model, vitamin D deficiency causes the expression of e-cadherin to be downregulated, resulting in loss of adhesion and a poorly differentiated state.[51] This occurs even in a triple-negative, metastatic breast cancer cell line, and results from demethylation of a promoter for e-cadherin biosynthesis.[52] Another study found that downregulation of e-cadherin was a necessary condition for metastatic overgrowth of breast cancer cell lines.[53] Expression of e-cadherin may be highly regulated by 25(OH)D concentration.[51] High levels of circulating 25(OH)D provide substrate for conversion to $1,25(OH)_2D$ that is synthesized via hydroxylation of 25(OH)D by the 1a hydroxylase.[54] Although the principal site of this synthesis is the kidney, 1a hydroxylase is produced in a wide range of tissue, including breast epithelial tissue.[54] $1,25(OH)_2D$ locally synthesized in breast epithelium is free to bind with the nuclear vitamin D receptor (VDR), unmasking the portion of the DNA that codes for assembly of e-cadherin.[51,52]

In the second phase of the model, Initiation, DNA is modified either through uncorrected errors that occur during replication or through exposure to mutagens such as ionizing radiation or free radicals.[50] These changes in the DNA, especially changes that occur in an environment in which cells are poorly differentiated, set the stage for malignancy and unchecked cell division.

The next phase is Natural Selection. In this phase, due to the operation of evolutionary forces, malignant cells with even a 1% competitive growth advantage will eventually overtake a tissue compartment.

In the Overgrowth phase, tumor cells grow outside the basement membrane of the tissue compartment in which they originated due to increasing scarcity of essential resources, such as oxygen and glucose, that are necessary for further growth and cell division.

As the tumor continues to grow, a few malignant cells will break off from the tumor mass and be transported by the lymphatic system or bloodstream where they will colonize remote tissue sites. This is known as the Metastasis phase. During the next phase, Involution, the growth of the tumor mass is temporarily halted by a seasonal rise in serum 25(OH)D concentration. This is supported by research that has demonstrated that diagnosis for breast cancer is highest in winter when population serum 25(OH)D levels are lowest.[55]

Under the vitamin D-cancer prevention hypothesis, this process can be stopped at almost any point in the DINOMIT model by restoring vitamin D sufficiency in the organism. Beyond the DINOMIT model, evidence from laboratory studies has demonstrated a powerful anti-cancer effect of vitamin D metabolites on three critical phases in the development of a breast tumor: differentiation, apoptosis, and angiogenesis.[23] Therefore, because vitamin D exerts such a powerful effect over a broad spectrum of processes essential for the development of a breast neoplasm, the criterion for a biological plausibility is well satisfied.

Consideration of Alternative Hypotheses

There are several well established risk factors for development of breast cancer. These include alcohol consumption,[56] exogenous estrogen,[57] ionizing radiation[58] and in postmenopausal women, obesity.[58] Obesity is associated with lower risk of premenopausal breast cancer, but higher risk of postmenopausal breast cancer.[59] Physical activity is another possible factor that might be related to sunlight and time spent out of doors.[60-62]

Studies have also demonstrated a protective effect of physical activity on risk of breast cancer.[63] However, it is difficult to separate the effect of physical activity from that of serum 25(OH)D concentration. Much of the physical activity may have been performed outdoors, and epidemiological investigations of the effect of physical activity on cancer risk rarely differentiate between indoor physical activity and outdoor physical activity. Furthermore, obesity is independently associated with low serum 25(OH)D. A reduced capacity to produce 25(OH)D in obese persons has been found in previous studies.[64] Interestingly, in studies performed by Bertone-Johnson et al., Crew et al. and Engel et al., serum 25(OH)D concentration was significantly, inversely associated with breast cancer risk after controlling for physical activity.[15,16,19]

According to a recent meta-analysis of studies on the relationship between alcohol consumption and breast cancer risk, excess risk associated with alcohol consumption was estimated to be approximately 22%.[65] This leaves a large amount of excess risk unexplained. Although a possible association between red meat consumption and breast cancer incidence has been investigated, the evidence from these investigations is inconclusive.[66] Yet

another risk factor that was thought to modify breast cancer risk is intake of dietary fat, theoretically by modifying levels of endogenous estrogen. However, in the Women's Health Initiative study population, there was no effect of a low fat diet on risk of breast cancer.[67] While exogenous estrogen in the form of hormone replacement therapy (HRT) increases risk of breast cancer in postmenopausal women,[68] use of HRT has declined substantially since 1993, when recommendations against use of HRT were widely disseminated.[69] It seems unlikely that use of HRT could account for the majority of breast cancer cases that occur every year.

This should probably be considered one of the weakest of Hill's criteria because in the face of strong epidemiologic evidence supporting a causal relationship between a disease and exposure of interest, the presence or lack of alternative hypotheses may be largely irrelevant. None of the above risk factors can unilaterally account for all the variation between individuals in breast cancer risk. Although these risk factors, when taken together, could make a substantial contribution to predicting breast cancer incidence rates at the population level, they still cannot account for all of the differences in incidence between individual women. Exposure to the main determinant of circulating 25(OH)D concentration, UVB irradiance, tends to be ubiquitous at the population level and depends chiefly on latitude, culture, and health behaviors that are shared by large groups of people. Therefore, vitamin D status may be able to account for a greater proportion of excess risk for breast cancer than factors of lower prevalence in the population.

In previous case-control studies of serum 25(OH)D concentration and breast cancer risk, up to an 80% lower estimated risk of breast cancer was observed in subjects with the highest levels of serum 25(OH)D,[20] Based on data on US population serum 25(OH)D levels from the NHANES III study and risk estimates from case-control studies,[42] the estimated population attributable risk of vitamin D insufficiency could be as high as 70% for breast cancer. Results from studies on serum 25(OH)D and breast cancer risk have also demonstrated a clear dose-response relationship. In a recent meta-analysis,[27] data from 11 studies were used to estimate the dose-response curve.

Experiment

This criterion is satisfied by a randomized controlled trial performed (RCT) by Lappe et al.[14] In this study, women in the treatment group received 1,100 IU of vitamin D_3 and 1,450 mg of calcium per day over 4 y. By the end of the 3 y follow-up period that started one year after beginning vitamin D and calcium, women in the treatment group experienced a 77% reduction in risk from all cancers (mainly lung, colon, and breast) compared with women in the placebo group ($p < 0.05$). A previous RCT using 400 IU/day of vitamin D_3 and 1000 mg of calcium observed a 4% reduction in breast cancer incidence, approximately the reduction expected from the known dose-response relationship.[34]

In addition, in a study performed by in the Women's Health Initiative, calcium and vitamin D intake significantly decreased the risk of total, breast, and invasive breast cancers by 14–20% in study participants who were not taking vitamin D or other supplements before enrolling in the study.[37]

Conclusion

Based on the current scientific evidence, vitamin D supplementation is an urgently needed, low cost, effective, and safe intervention strategy for breast cancer prevention that should be implemented without delay. There have been over 30 studies performed on toxicity of vitamin D. These studies have shown that at oral intakes of up to 10,000 IU per day of vitamin D_3 or serum 25(OH)D concentrations below 100 ng/ml, no adverse health effects have been observed.[70-72] In a randomized controlled trial of vitamin D with pregnant and lactating women, supplementation with 4,000 IU/d vitamin D_3 did not result in any adverse effects such as hypercalcemia or hypercalcuria.[73] Moreover, the Institute of Medicine recently established 4,000 IU per day as the tolerable upper limit of safe intake.[74]

Additional epidemiological studies of the effect of high serum concentrations of doses of vitamin D on breast cancer risk should be performed. Randomized controlled trials (RCT) of oral intake of 4,000 IU/day of vitamin D_3, with separate trials for premenopausal and post-menopausal women residing at latitudes > 37 degrees north are one option. However, epidemiological history has shown that an RCT is not necessary to establish causality or to prevent a disease. For example, it is widely accepted that tobacco smoking causes lung cancer,[75] yet this knowledge was gained as the result of ordinary observational studies. Examples of this type abound in the history of epidemiology, such as John Snow's use of an ecological approach to elucidate the cause of cholera,[76] or use of contact tracing for tuberculosis.[77] Furthermore, RCT's take far longer to complete and can cost up to 350 times as much as a nested case-control or ordinary case control study of the same topic when the purpose is testing prevention.

Study after study, utilizing varying designs in both human populations and the laboratory, has demonstrated that vitamin D substantially reduces the risk of breast cancer. The A.B. Hill criteria have been largely satisfied, providing a compelling case for a causal, inverse relationship between vitamin D status and risk of breast cancer.

Disclosure of Potential Conflicts of Interest

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