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Insulin use, adipokine profiles and breast cancer prognosis

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ABSTRACT

Background: Type-2 diabetes mellitus (T2DM) and breast cancer (BC) share common cytokine signaling changes resultant from adipose tissue dysfunction. This modified adipokine signaling was shown to be directly associated with changes in the body mass index (BMI) and diet and it is expected to also be influenced by T2DM pharmacotherapy. We evaluated the relationship between pre-existing diabetes treatment, circulating adipokine levels at cancer diagnosis, and long-term outcomes.

Methods: All incident BC cases were reviewed (01/01/2003-12/31/2009, N = 2194). Each of the subjects with baseline T2DM (cases) was matched with two other subjects without T2DM (controls) based on the following criteria: age, BMI, ethnicity, menopausal status and tumor stage. All cases and controls with available baseline plasma and tumor biopsies, and being surgery and BC treatment naïve, were included ($N_1 = 97$, $N_2 = 194$). Clinical history and vital status were documented. Adipokine levels (adiponectin, leptin, TNF-α, CRP, IL-1β, IL-1Ra, IL-6, and C-peptide) were assessed by either ELISA or Luminex® assays. Cancer outcomes were assessed by Kaplan-Meier analysis; associations between categorical variables were assessed by Fisher's exact test, categorical and continuous variables by Kruskal-Wallis or Wilcoxon Rank-Sum test, where appropriate. Multivariate adjustments (MVP, multivariate p-value) were performed accounting for age, tumor stage, BMI, estrogen receptor (ER) status and cumulative comorbidity. All biomarker correlations were assessed by the Pearson method.

Utilization of insulin and insulin secretagogues was associated with ER (−) phenotype ($p = 0.008$, $p = 0.043$) and poorer BC outcomes ($p = 0.012$, $p = 0.033$). Insulin users were found to have lower C-peptide and higher IL-6, TNF-α and CRP levels, of which elevated CRP and TNF-α were associated with poorer BC outcomes ($p = 0.003$, MVP = 0.210). Insulin remarked by higher leptin levels as compared to controls ($p = 0.052$), but did not differ significantly from non-users. Although lower adiponectin levels were observed among non-insulin users as compared to controls ($p < 0.001$, MVP = 0.006), insulin use seemed to have restored adiponectin production. C-peptide levels were lower among insulin users as compared to non-users ($p < 0.001$, MVP < 0.001) and approached levels comparable with those of the controls. In the overall dataset, C-peptide lower than 0.75 ng/ml were strongly associated with poorer survival ($p = 0.007$, MVP = 0.002). Among insulin users, C-peptide levels were inversely correlated with IL-1β and IL-1Ra levels only after full adjustment ($p = 0.012$, $p = 0.030$); the correlation was unremarkable in other groups.

Conclusion: Insulin use is associated with elevated leptin, CRP, TNFα, and lower C-peptide and also linked to poor BC outcomes. More research is needed to verify these findings; however, we are among the first to correlate pharmacotherapy use, measures of adipose tissue dysfunction and cancer outcomes.

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1. Introduction

A hallmark of type-2 diabetes mellitus (T2DM), hyperinsulinemia was identified as an independent breast cancer (BC) risk factor that has also been associated with more advanced cancer stages at diagnosis and poorer prognosis [1,2]. Recognized today as an immune-mediated disease, T2DM is thought to be triggered by adipose tissue dysfunction [3]. The same adipocyte impairment is believed to be responsible for the occurrence of the low-grade, chronic inflammation associated with the development of BC [4]. Given these facts, it is hard to overlook the evidence that adipocytes represent a vast majority of the breast tissue [5] and that adipocyte-produced signaling molecules - adipokines - have been identified as potential BC prognostic indicators [6,7]. All together, these findings raise the question of whether or not the physiologic conditions leading to the progression of T2DM and those involved in the occurrence of BC are, in fact, related. If true, then T2DM management, particularly pharmacotherapy, exercise and diet, might be responsible for the changes in adipokine production and occurrence of an environment more accommodating for BC growth. Our group and others have indicated that T2DM pharmacotherapy plays a significant role in the physiological changes that subsequently lead to cancer progression [8–10]. Although efforts have been made to evaluate adipokine changes in patients with BC receiving metformin [11], the evidence connecting specific T2DM pharmacotherapies with adipokine changes and BC outcomes is lacking. Based on our knowledge, this study is the first to report specific correlations between adipokine profiles, pharmacotherapy and BC outcomes.

Adipocytes - along with their autocrine, paracrine and endocrine functions - are exceptionally positioned to modulate breast tumorigenesis and BC development [12]. In the context of obesity-induced adipose tissue dysfunction, T2DM is accompanied by profound changes leading to increased BC risk and poorer prognosis [13]. While the exact process leading to impaired insulin sensitivity is not entirely elucidated, numerous adipocyte-derived cytokines, such as interleukins 1β and 6 (IL-1β, IL-6), tumor necrosis factor alpha (TNF-α), as well as C-reactive protein (CRP) have been consistently reported in association with T2DM occurrence and hyperinsulinemia [14–16]. Recent evidence suggests that this may be due to the macrophage infiltration of dead adipocytes and resulting hypoxia due to poor vascularization of the adipose deposits [17]. A very similar biomarker pattern was observed in advanced BC stages [18,19], suggesting that the common ground linking the pathophysiology of T2DM and BC is most likely the adipocyte dysfunction and an unbalanced adipokine profile. A summary of the evidence contributing to the understanding of the relationship between adipokine production, T2DM and BC is presented in Table 1.

Following the same paradigm, but far more interesting, is the implied possibility that these physiologic conditions ultimately leading to BC could be altered by the *selection* of T2DM pharmacotherapy. In this hypothesis the utilization of exogenous insulin, as well as of other drugs that stimulate the secretion of endogenous insulin, are expected to trigger the same BC risk as naturally-occurring hyperinsulinemia in obesity, a process driven by the adipocyte dysfunction. This rationale is well supported by the recent literature indicating that insulin use in patients with T2DM has been associated with higher BC incidence and mortality [20–22]. Although these studies did not expand into the mechanism by which T2DM pharmacotherapy modified the BC outcomes, it seems plausible that certain diabetes pharmacotherapies - such as injectable insulin or oral therapies stimulating endogenous insulin production (secretagogues) - could have impacted the adipocyte metabolism and adipokine production which, in turn, led to worsening of the BC outcomes.

The present case-control study aimed at filling the above-described gap of evidence by comparing baseline adipokine profiles in a control-matched group of women with or without T2DM and newly diagnosed BC. The investigation presented here was undertaken to evaluate overall survival (OS) and disease-free survival (DFS) in relationship with T2DM pharmacotherapy utilization and, at the same time, with the adipokine profiles at the time of BC diagnosis. For T2DM treatment reference, Table 2 provides a

Table 1
Adipokine levels in obesity, T2DM, and BC and relationships with BC.

Biomarker	Normal levels	Levels in T2DM	Levels in BC	Levels in obesity	Relationship with BC
Adiponectin (μg/ml)	3.5–22.4* [23] 11.7 ± 1.0 [24] 8.83 ± 0.38 [25] 10.2 + 4.3 [26] 13.3 ± 1.8 [27] 25.55 ± 6.1 [28]	7.6 ± 0.7 [24] 5.5 ± 1.6 [26]	7.57 ± 0.31 [25]	6.1 ± 2.0[26] 8.6 ± 0.8[27]	Usually ↓ in BC [29] Suppress ER (−) BC [30] ↓ level ↑ BC risk [25] ↓ BC proliferation [31]
Leptin (ng/ml)	5.9 ± 0.7 [27] 21.47 ± 16.9 [28] 6.0 ± 1.7 [32] 6.3 ± 3.1 [33]	18.3 (6.3–57.8) [34] 33.0 ± 5.6 [32]	90.3 ± 27.5 (μM) [35]	26.9 ± 3.9 [27] 37.1 ± 32.6 (μM) [35] 54.9 ± 4.5 [32] 52.8 ± 24.6 [33]	↑ in BC [35]
CRP (μg/ml)	1.2 ± 0.3 [32] 3.18 ± 3.00 [36] 1.33 (0.60–3.33)† [37] 2.6 (1.0–6.1)† [38] 2.45 ± 4.38 [39]	5.8 ± 1.1 [32] 2.68 (1.28–4.94)† [37] 6.9 (4.2–10)† [38] 4.14 ± 5.1 [39]	4.5 ± 8.3 [40]	6.3 ± 1.1 [32] 1.35 (0.57–2.18)† [41]	↑ level ↑ BC risk [40,42]
IL-6 (pg/ml)	0.39 ± 0.06 [32] 1.38 (0.91–2.05)† [38] 1.67 ± 1.59 [39] 0.5 ± 0.4 [43] 1.28 ± 0.85 [44] 1.28 ± 0.85 [44]	3.58 ± 0.51 [32] 2.00 (1.43–2.78)† [38] 2.45 ± 1.80 [39]	9.1 ± 19.5 Locoregional: 1.4 ± 1.4 Metastatic: 12.4 ± 22.5 [43]	2.78 ± 0.3 [32] 2.19 (1.18–4.40)† [41] 7.69 ± 5.06 [44]	↑ BC progression [42] ↑ in metastatic BC [43] ↑ in depressed BC [45]
TNFα (pg/ml)	0.74 ± 0.09 [32] 1.79 ± 1.28 [39] 1.72 ± 0.26 [46] 0.98 ± 0.37 [47]	1.08 ± 0.12 [32] 2.04 ± 1.51 [39] 1.87 ± 0.31 [46] 5.8 ± 1.7 [48]	1.47 ± 0.58 [47] 15.9 ± 0.9 [48]	1.48 ± 0.15 [32] 3.65 (2.98–4.53) [41]	↑ poor prognosis [47] ↓ post complete response [48]
IL-1β (pg/ml)	0.47 ± 0.79 [39] 6.22 + 11.9 (no units) [49] 5.81 ± 1.80 [50]	0.57 ± 0.93 [39]	27.3 ± 75.5 (no units) [49]	12.34 ± 3.70 [50]	↑ leptin expression [51] ↑ ER transcription [52]

Values at mean ± SD, *range, or †median (interquartile range).