

ORIGINAL ARTICLES

Relationship between salivary adiponectin, IGF-1, obesity and breast cancer

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ABSTRACT

Objective: The objective of this study was to determine if adiponectin and IGF-1 salivary concentrations are altered in combination with the presence of obesity and breast cancer. The null hypothesis is that there are no significant adiponectin and IGF-1 concentration alterations secondary to the presence of obesity and/or carcinoma of the breast.

Methods: There were two groups of test subjects: healthy controls (n = 20) and individuals diagnosed with breast cancer (n = 20). The two cohorts were further stratified into four groups. These included subjects who are healthy and of normal BMI (n = 10); are healthy but have an elevated BMI (n = 10); have breast cancer and a normal BMI (n = 10); and have cancer and an elevated BMI (n = 10). The presence and concentration of adiponectin and IGF-1 was determined using the ELISA methodology.

Results: The investigation revealed a significant increase in mean adiponectin levels in subjects with cancer compared to the controls ($t = -2.57$; $p < .01$). Individuals that were diagnosed with breast cancer and were obese exhibited the highest concentrations ($F = 5.13$; $p < .005$) of adiponectin. Adiponectin concentrations were also found to be correlated to IGF-I levels ($r = 0.05$; $p < .001$).

Conclusion: Salivary adiponectin levels were significantly higher among cancer group. There were no significant differences between the cancer and control groups for IGF-I levels.

Key Words: Adiponectin, IGF-1, Breast cancer, Saliva, Obesity

1. INTRODUCTION

Obesity is a chronic disorder which is reaching epidemic proportions throughout the United States.^[1] Nearly two-thirds of the American population are either overweight or obese.^[1] Additionally, obesity is a risk factors for developing numerous systemic illnesses such as type-2 diabetes, cardiovascular disease and among women, breast cancer. Obesity is, indeed, a multifactorial disorder; however, the dysregulation of adipokines or hormones may be the underlying cause for the aforementioned diseases. Two proteins gaining attention in this area of research are adiponectin and insulin

growth factor-1 (IGF-1).^[2-4]

Adiponectin, an adipose tissue-specific peptide hormone involved in glucose metabolic pathways, increases insulin sensitivity by decreasing circulating fatty acid levels.^[2] Interestingly, serum levels of adiponectin decrease linearly with an increase in BMI, percent body fat, visceral fat, subcutaneous abdominal fat, and central fat distribution, even though it is produced by adipose tissue.^[3,4] Similarly, breast cancer risk in both premenopausal and postmenopausal women is inversely related to serum adiponectin levels. This relation-

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ship is much stronger in postmenopausal women because they have low levels of estradiol, a negative determinant of adiponectin.^[4-6] It would follow that obese, postmenopausal women have low serum adiponectin and are more likely to develop breast cancer. Women with very low adiponectin levels are more likely to develop larger and more aggressive tumors.^[7]

Insulin-like growth factor-1 (IGF-1) is a 7.6 kDa polypeptide with a variety of complex functions. It has both endocrine and paracrine functions that regulate tissue growth, metabolism and cell differentiation.^[8,9] A DNA polymorphism near the promoter region of the IGF-I gene has been associated with serum IGF-I levels, body height, birth weight, growth and central obesity. An environment that promotes obesity leads to a more pronounced fat accumulation in variant carriers of IGF-I polymorphisms.^[10,11] IGF-I seems closely related to growth hormone, possibly as a regulator, so much so that some consider it an overall indicator of growth hormone.^[9] Similar to adiponectin, IGF-I is largely produced by adipose tissue, studies on the relationship between IGF-I and obesity conflict.^[12] Some show that circulating IGF-I increases with BMI,^[13] while others show no significant correlation or a decrease in circulating IGF-I.^[14-16] Both IGF-I and growth hormone increase in research observing individuals going through the weight-loss process from either gastric bypass surgery or exercise and diet changes.^[8,17] IGF-I is an indicator of high risk of disease, as well. Low serum IGF-I indicates a higher risk of ischemic heart disease, while high serum IGF indicates an increased risk of developing prostate or breast cancer.^[18-20] Additionally, an association between IGF-I levels and the pathogenesis of cancer has been implicated in colorectal cancer and ovarian cancer.^[21-23]

IGF-I, in association with leptin, are also related to physical activity levels, which influence the prognosis of cancer patients. Obese and physically inactive breast cancer patients may have poorer survival rates when compared to lighter weight and women that are more active. High leptin levels were found with elevated body mass index; therefore, increasing physical activity and decreasing body fat may be a reasonable intervention approach to change leptin levels, thereby potentially influencing breast cancer prognosis.^[24]

Current studies show that many of the same diagnostic markers for breast cancer that are found in serum, plasma and cancer tissues are also found in saliva.^[25] Tumor markers c-erbB-2 and cancer antigen 15-3 are elevated in the saliva of cancer patients. In addition, protein concentrations of the tumor suppressor oncogene protein p53 are low in the saliva of cancer patients. These findings are consistent with similar elevations of the same markers in plasma. Saliva composition

also gives an accurate presentation of the current conditions of the body – a real-time image of the levels of such proteins in the body. This makes saliva a good diagnostic tool for investigating breast cancer progression.^[25] Therefore, the purpose of this study is to see if adiponectin and IGF-1 are present and altered in a diseased state, such as obesity and/or breast carcinoma. The null hypothesis is that there are no significant analyte differences between healthy individuals and the diagnosed with obesity and/or state, carcinoma of the breast.

2. METHODS

2.1 Participants

This study was performed under the auspices of UTHSC IRB approved protocol# HSC-DB-05-0394; whereby, saliva samples were obtained from two groups of volunteers, each consisting of twenty women. All participating volunteers were explained their participation rights and signed an IRB consent form. Afterwards, a saliva specimen and medical data were collected from the participant's medical record. All participants were administered a questionnaire at the time of signing the IRB approved consent form. The questionnaire obtained socio-economic data along with information concerning tobacco and alcohol usage. The medical data was collected from the medical record, which included information concerning their pharmacological, gynecological and medical histories.^[26] The menopausal status was self-reported and the hysterectomies reported in this study were hysterectomies with bilateral salpingo-oophorectomy. The related clinical data and corresponding saliva specimens were non-linked and bar coded in order to protect patient confidentiality. Patients were consecutively selected as they entered the oncology center and their health status i.d., healthy vs. cancer was histologically determined by the pathology report. Tumor staging and nodal status were assessed according to the criteria set forth by the American Joint Committee on Cancer.^[27] All information concerning the cancer cohort was obtained prior to treatment.

2.2 Body mass index calculations

Body mass index calculations (BMI) were performed according to the NHLBI/NIH guidelines using the following formula: $BMI = \text{Height}/(\text{weight})^2$ where height is expressed in meters and weight in kilograms. A BMI of greater than thirty was used to characterize an individual as obese.^[28]

2.3 Stimulated whole saliva collection

The participants were seen between 8 a.m. and 5 p.m. to control for circadian rhythms. The study required that the individuals not eat, drink, smoke, brush or rinse for at least 60 minutes prior to saliva collection. Stimulated whole saliva

(SWS) was performed by having the subject first swallow accumulated saliva in the mouth. An unflavored, unsweetened preweighed piece of chewing gum base was placed in the mouth by the volunteer and masticated (60 chews/min) as monitored with a metronome.^[25] Accumulated saliva was expectorated after each minute of chewing for a total of five minutes. The weight of gum base after collection was determined and physical characteristics were recorded. SWS is expressed as ml/minute. Collected specimens were centrifuged to remove unwanted particulates. Afterwards, the supernants were separated from the pellet and immediately frozen (-80°C) until analysis.

2.4 Laboratory methods

The concentration of adiponectin and IGF-1 were determined using the enzyme-linked immunosorbent assay (ELISA) method. Both ELISA assay kits were purchased from R&D Systems[®] and used according to the manufacturer's instructions. The saliva specimens were blindly assayed. Each specimen was thawed until it reached room temperature.^[26] The assays for both analytes were performed concurrently in order to control for the "freeze-thaw" effect.

2.4.1 Adiponectin ELISA determinations

Briefly, standards were prepared by reconstituting the standard in calibrator diluent and by performing a dilution series producing concentrations of 250, 125, 62.5, 31.2, 15.6, 7.8, and 3.9 ng/ml. The calibrator diluent was used as the zero standard. All standards and samples were run in duplicate. 50 μ l of standard and sample were applied directly to the appropriate wells and left overnight in a refrigerator at three degrees Celsius. The wells were then aspirated and washed four times with wash buffer. The plate was inverted and blotted to remove excess wash buffer. 200 μ l of adiponectin conjugate was added to each well and the plate was allowed to incubate at room temperature for two hours. Afterwards, the plate was washed four times. Equal parts of color reagents A and B were mixed to provide 200 μ l of substrates solution for each well. Substrate solution was added and the plate incubated at room temperature for thirty minutes. 50 μ l of stop solution (2N sulfuric acid) was added to each well. The optical density of the wells was determined using a microplate reader set at a wavelength of 450 nm. Adiponectin concentrations are expressed in ng/ml.

2.4.2 IGF-1 determinations

IGF-1 standards were prepared by reconstituting the standard in a calibrator diluent and by performing a serial dilution resulting in standard concentrations of 6, 3, 1.5, 0.75, 0.375, 0.188 and 0.094 ng/ml. The calibrator diluent was used as the zero standard. All standards were run in duplicate. All samples were run in triplicate. 50 μ l of standard and

sample were applied directly to the appropriate wells and left overnight in a refrigerator at three degrees Celsius. The wells were aspirated and washed four times with buffer. The plate was inverted and blotted to remove excess wash buffer. 200 μ l of IGF-1 conjugate was added to each well and the plate was allowed to incubate in the refrigerator at three degrees Celsius for one hour. The plate was washed four times. Equal parts of color reagents were mixed to provide 200 μ l of substrate solution for each well. Substrate solution was added and the plate incubated at room temperature for thirty minutes, protected from light. 50 μ l of stop solution (2N sulfuric acid) was added to each well. The optical density of the well contents was determined, using a microplate reader, set to 450 nm. IGF-1 concentrations are in ng/ml.

2.4.3 Assay quality assurance

For all their power, immunoassays are subject to many kinds of interference. Quality control tests were performed to control for these problems. With respect to ligand recovery, we were able to establish the amount of marker (ligand) recovered from saliva samples. Five saliva specimens with known amounts of marker were serially diluted. The dilutions were assayed for both markers. The data were plotted against the expected values to determine the linearity of dilution. The slopes of both the dose-response curve and the standard curve were not significantly different from each other, and the intercepts were not significantly different from zero. During the assaying of the specimens, the investigators employed the use of appropriate positive and negative controls for all marker assays. When performing the assays, some test specimens contained primary antibodies preincubated with excess ligand to control for false-positives. In addition, test specimens were preincubated with excess free primary antibody to determine if the signal had been eliminated. These extra tests provided additional quality control during the course of specimen analyses.

2.5 SWS western blotting

After a review of the data from the ELISA analysis, it was decided to assay adiponectin using a different methodology in order to confirm the findings. Therefore, a western blot was performed for adiponectin across the varying stages of breast cancer. The western blot was not performed for IGF-1 as the protein did appear to be a major factor in the overall analysis. The saliva specimens for each stage of cancer were pooled with the exception of stage IIa, which contained only one subject. The healthy group consisted of a pool of subjects that were healthy and not obese. A benign tumor specimen served as a positive control. The saliva pools' protein concentration was analyzed using a Bradford assay.

To each saliva pool, an equal volume of Laemmli sample

buffer containing 5% B-mercaptoethanol was added. The samples were then heated in a 95°C dry heat block for five minutes. The samples were then loaded onto a Tris-HCL polyacrylamide gel. The following percentage gel was used depending on the size of the protein being tested. In this case was 12% was appropriate for adiponectin.

The gels were run at 100 volts for approximately one hour in the Mini-PROTEAN 3 Cell (Bio-Rad). The gels were transferred to Immuno-Blot PVDF membrane (Bio-Rad Laboratories, Hercules CA) in a 1× Tris-Glycine Buffer containing 20% Methanol using the Trans-Blot SD semi-dry transfer cell (Bio-Rad Laboratories, Hercules CA) at 200 mA for 45 minutes. The membranes were blocked for four hours at 2°C-8°C with agitation using 5% blotting grade blocker (Bio-Rad Laboratories, Hercules CA) in 1× phosphate buffered saline solution with tween detergent (PBST). The primary antibody was diluted in 20 ml of 5% blocker/PBST and added to the membrane. The primary antibody for adiponectin was diluted to a 1:1000 solution.

The hybridization reaction was performed at 2°C -8°C overnight with agitation. After hybridization, the membranes were washed three times with 5% blocker/PBST buffer for ten minutes each at room temperature. The secondary antibody used for adiponectin, was goat anti-mouse-HRP conjugate (Bio-Rad Laboratories, Hercules CA) at 1: 10,000 dilution. The secondary antibody was diluted in 10 mls of 5% blocker/PBST and added to the membranes. The hybridization reaction was performed at room temperature for one hour with agitation. Three ten minute washes in 1× PBST were performed.

The membranes were next subjected to a chemiluminescent detection using a solution containing luminol (Sigma), p-coumaric acid (ICN Biomedicals, Inc., San Francisco, CA), and hydrogen peroxide (Fischer Scientific, Hampton, NH). Afterwards, 22 µl p-coumaric acid, 50 µl luminol, and 3 µl hydrogen peroxide was added to 10 mls of 100 mM Tris-HCL, pH 8.5. The membranes were mixed with the luminol solution for 45 s, placed in a plastic sleeve, and exposed to Fuji Super RX autoradiography film for one to five minutes.

2.6 Statistical analysis

Statistical analyses were performed using the SPSS™ statistical software package. Three levels of statistical analyses were performed on the data. Initially, descriptive analyses were made for the group of individuals with respect to adiponectin and IGF-1 concentrations and BMI. Descriptive analyses were also performed for the various tumor pathology subgroups with respect to analyte concentrations. Cross-tabulation tables were performed for frequency deter-

minations among the subgroups.

The second level of analysis was to determine if there were any associations among specific variable. The Spearman rho correlation was executed to determine associations between the continuous or the scale variables IGF-1, adiponectin concentrations and BMI. The Pearson's Point-Biserial Correlation Coefficient was used to determine associations between the nominal variable (health status) and continuous variables.

The third level was performed to compare mean values for the various subgroups. The Student's *t* test was used to make bivariate analysis. For multivariate analysis, the mean values were compared using a one-way analysis of variance. If the overall ANOVA is found to be significant, then a Tukey post-hoc analysis was performed.

Table 1. The mean values and standard deviations for Adiponectin, IGF-1 and BMI for anthropometric and social variables

Characteristic	Status	n	Mean (± Std. Dev.)	Significance
Clinical status				
Height in meters	Healthy	20	1.65 (± 2.40)	n.s.
	Cancer	19	1.59 (± 0.06)	
Weight in kg	Healthy	20	70.97 (± 42.61)	n.s.
	Cancer	19	84.74 (± 74.13)	
Body mass index	Healthy	20	26.29 (± 7.60)	n.s.
	Cancer	20	35.53 (± 16.37)	
Race				
Height in meters	Caucasian	24	1.65 (± 0.06)	n.s.
	Afro American	15	1.59 (± 0.16)	
Weight in kg	Caucasian	24	71.38 (± 21.47)	n.s.
	Afro American	15	87.75 (± 33.58)	
Body mass index	Caucasian	24	26.78 (± 8.78)	<i>t</i> = -2.58; <i>p</i> < .04
	Afro American	16	37.10 (± 16.82)	
Adiponectin	Caucasian	24	8.71 (± 13.51)	n.s.
	Afro American	16	19.28 (± 37.67)	
IGF-1	Caucasian	24	0.21 (± 0.15)	n.s.
	Afro American	16	0.19 (± 0.21)	
Tobacco usage				
Body mass index	Tobacco non-user	29	28.87 (± 10.02)	n.s.
	Tobacco user	11	36.30 (± 19.47)	
Adiponectin	Tobacco non-user	29	14.32 (± 29.42)	n.s.
	Tobacco user	11	9.31 (± 14.55)	
IGF-1	Tobacco non-user	29	0.22 (± 0.19)	n.s.
	Tobacco user	11	0.15 (± 0.09)	
Alcohol consumption				
Body mass index	Non user	15	33.58 (± 12.86)	n.s.
	1 or more/week	13	28.28 (± 14.78)	
Adiponectin	Non user	15	15.83 (± 36.47)	n.s.
	1 or more/week	13	12.80 (± 19.21)	
IGF-1	Non user	15	0.19 (± 0.13)	n.s.
	1 or more/week	21	0.23 (± 0.23)	

3. RESULTS

The cohorts were numerically balanced with twenty individuals in both the healthy and cancer groups. The mean age for the healthy control group was 47.7 (± 11.0) years while the cancer cohort was 50.3 (± 11.0) years. Likewise, the stratified groups for BMI status were numerically balanced with ten individuals in each cohort. There were ten volunteers in the healthy/normal BMI (HNB), healthy/obese BMI

(HOB), cancer/normal BMI (CNB) and cancer/obese BMI (COB) groups. However, racial differences were not equally distributed across the four groups. The totals for Caucasians across the four groups is 8, 9, 3 and 4 respectively; whereas, the totals for African-Americans across the four groups is 2, 1, 7, and 6 respectively.

The clinical and social parameters for both cohorts are illustrated in Table 1. Of these variables, only BMI exhibited a significant for racial differences. BMI was significantly higher for African Americans as compared to Caucasians ($t = -2.58$; $p < .04$). IGF-1 was not significant across the variables listed in Table 1.

Table 2 represents the mean values and standard deviations for Adiponectin, IGF-1 and BMI for gynecological variables. There were no mean differences for these variables except for menopausal ($t = -1.97$; $p < .059$) and hysterectomy ($t = -1.99$; $p < .05$) status with respect to adiponectin concentrations. Premenopausal women had significantly lower adiponectin levels as well those that did not have a total hysterectomy. Additionally, IGF-1 levels were not significant for the gynecological variables.

As illustrated in Table 3, the *t*-test analyses for mean comparisons presented that the adiponectin concentrations ($t = -2.57$; $p < .01$) and BMI ($t = -2.245$; $p < .03$) were significantly higher for the cancer group as compared to the healthy controls.

A one-way analysis of variance was executed to compare mean adiponectin differences between the four groups of varying BMI status i.e., Healthy BMI, Healthy-Obese BMI,

Cancer Normal BMI and Cancer Obese BMI. The overall model was significant across the four BMI groups ($F = 5.13$; $p < .005$). The Tukey post-hoc analysis exhibited a significantly higher mean value the obese cancer group than the Healthy BMI ($p < .01$), Healthy-Obese BMI ($p < .01$) and Cancer Normal BMI ($p < .05$).

Table 2. Means and standard deviations for gynecological variables

Variable	Status	n	Mean (± Std. Dev.)	Significance
Birth control pill usage				
Body mass index	No	27	31.83 (± 14.71)	n.s.
	Yes	9	29.54 (± 10.88)	
Adiponectin	No	27	16.06 (± 30.35)	n.s.
	Yes	9	7.85 (± 14.70)	
IGF-1	No	27	0.19 (± 0.18)	n.s.
	Yes	9	0.22 (± 0.14)	
Menopausal status				
Body mass index	Premenopausal	17	31.94 (± 16.35)	n.s.
	Postmenopausal	23	30.15 (± 11.14)	
Adiponectin	Premenopausal	17	4.82 (± 10.21)	$t = -1.97$; $p < .059$
	Postmenopausal	23	18.94 (± 32.29)	
IGF-1	Premenopausal	17	0.19 (± 0.16)	n.s.
	Postmenopausal	23	0.21 (± 0.19)	
Hormone replacement				
Body mass index	No	22	31.48 (± 12.91)	n.s.
	Yes	14	28.28 (± 8.23)	
Adiponectin	No	22	13.61 (± 30.13)	n.s.
	Yes	14	14.28 (± 23.49)	
IGF-1	No	22	0.18 (± 0.15)	n.s.
	Yes	14	0.27 (± 0.22)	
Hysterectomy performed				
Body mass index	No	26	28.70 (± 10.36)	n.s.
	Yes	12	37.03 (± 18.21)	
Adiponectin	No	26	7.75 (± 13.27)	$t = -1.99$; $p < .05$
	Yes	12	25.60 (± 41.9)	
IGF-1	No	26	0.19 (± 0.16)	n.s.
	Yes	12	0.24 (± 0.21)	

Table 3. Means and standard deviations across cancer and obesity status

Status	n	Age	Adiponectin	IGF-1	BMI	SWS
Healthy	20	48	3.08 (± 3.74)	0.20 (± 0.16)	26.3 (± 7.60)	1.40 (± 0.35)
Cancer	20	51	22.91 (± 34.28)*	0.20 (± 0.19)	35.5 (± 16.37)**	1.42 (± 0.91)
Healthy BMI	10	47	4.47 (± 4.77)	0.27 (± 0.18)	20.3 (± 0.70)	1.30 (± 0.34)
Healthy-obese BMI	10	49	1.69 (± 1.58)	0.13 (± 0.09)	32.3 (± 6.50)	1.50 (± 0.34)
Cancer normal BMI	10	49	8.82 (± 14.35)	0.13 (± 0.08)	21.8 (± 2.30)	1.67 (± 1.07)
Cancer obese BMI	10	53	37.01 (± 26.08)	0.27 (± 0.24)	48.9 (± 12.3)***	1.16 (± 0.68)

Note. *Adiponectin ($t = -2.57$; $p < .01$); **BMI ($t = -2.245$; $p < .03$); *** Cancer obese BMI > Healthy ($p < .01$), Healthy-obese ($p < .01$), Cancer normal ($p < .05$).

Additionally, a Spearman’s rho correlation analysis was implemented to determine if there was any relationship between, BMI, Adiponectin and IGF-1. The analysis revealed a moderate association between Adiponectin and IGF-1 ($r^2 = 0.25$, $r = 0.50$, $p < .001$). The Pearson’s Point-Biserial Correlation Coefficient was used to determine associations between the nominal variable (health vs. cancer) continuous variables. The analysis revealed a moderate positive association be-

tween health status and adiponectin ($r = 0.38$, $p < .02$) and BMI ($r = 0.3$, $p < .04$).

The break down for adiponectin and IGF-1 across pathological subtypes and receptor status are shown in Tables 4 & 5 respectively. Among the twenty volunteers within in the cancer cohort, there were two individuals diagnosed with ductal carcinoma in situ, thirteen with infiltrating ductal carcinoma, three with lobular carcinoma in situ and two with infiltrating

lobular carcinoma. Twelve individuals had no lymph node involvement while eight were node positive (N1 = 5; N2 = 3). None of the individuals had metastatic breast disease. There were twelve individuals with tumors less than 20 mm and eight that were larger than 20 mm. The “T” values could not

be stratified for analyte concentrations due to the small sample size. Consequently, the “T” values were dichotomized into two groups. The two groups were “T” less than 20 mm and “T” greater than 20 mm.

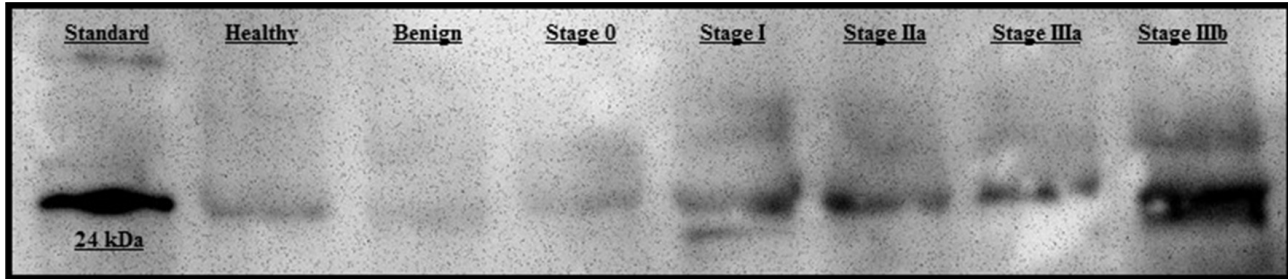


Figure 1. Represents a western blot of the cancer saliva samples pooled according to cancer staging

The ELISA results for adiponectin were crosschecked using a different technology, namely western blot. Figure 1 is a western blot of the pooled cancer specimens according to staging. A benign sample was added in the analysis as a positive control and the control was pooled from the non-obese healthy volunteers. As shown in Figure 1, Stage IIIb has the darkest band, which corresponds to the very elevated adiponectin concentration exhibited in Table 4.

Table 4. Illustrates the frequency and percentages for the pathological subtypes

Status	n	Adiponectin	IGF-1	BMI
Tumor staging				
Stage 0	2	0.34 (± 0.30)	0.09 (± 0.01)	18.95 (± 3.32)
Stage I	9	24.84 (± 28.52)	0.26 (± 0.26)	42.91 (± 17.21)
Stage IIA	1	3.68 (± 0.00)	0.10 (± 0.00)	23.2 (± 0.0)
Stage IIIA	5	9.40 (± 10.47)	0.13 (± 0.08)	21.8 (± 2.3)
Stage IIIB	3	60.35 (± 66.75)	0.20 (± 0.17)	48.9 (± 12.3)
Tumor size				
< 20 mm	12	18.95 (± 26.56)	0.22 (± 0.23)	38.85 (± 17.70)
> 20 mm	8	28.56 (± 45.03)	0.11 (± 0.11)	30.56 (± 13.70)
Node status				
Negative	12	21.18 (± 25.95)	0.23 (± 0.23)	36.57 (± 17.65)
Positive	8	25.22 (± 46.16)	0.18 (± 0.11)	32.49 (± 14.82)

As presented in Table 5, there were fifteen individuals assessed for estrogen, progesterone and Her2/neu receptors. Five were not assessed due to their small tumor size. With respect to estrogen, progesterone and Her2/neu receptors status, none of the adiponectin or IGF-1 concentrations was statistically significant across the receptor status variables; however, the estrogen and progesterone levels were nearly threefold higher for receptor positive status as compared to the receptor negative status. The reverse was true for Her2/neu receptor status where the receptor negative individuals were approximately three times higher for adiponectin concentrations.

Table 5. Mean values and standard deviations according to receptor status

Analyte	Receptor status	n	Mean (± Std. Dev.)
Estrogen			
Adiponectin	Negative	5	11.66 (± 18.02)
	Positive	10	29.13 (± 43.00)
IGF-1	Negative	5	0.17 (± 0.15)
	Positive	10	0.15 (± 0.06)
BMI	Negative	5	32.90 (± 15.54)
	Positive	10	33.55 (± 12.72)
Progesterone			
Adiponectin	Negative	8	11.35 (± 15.55)
	Positive	7	36.97 (± 49.69)
IGF-1	Negative	8	0.16 (± 0.12)
	Positive	7	0.16 (± 0.07)
BMI	Negative	8	28.76 (± 13.08)
	Positive	7	38.55 (± 12.04)
Her2neu			
Adiponectin	Negative	7	37.26 (± 49.45)
	Positive	8	11.10 (± 15.73)
IGF-1	Negative	7	0.14 (± 0.08)
	Positive	8	0.17 (± 0.11)
BMI	Negative	7	34.29 (± 12.99)
	Positive	8	32.49 (± 14.15)

4. DISCUSSION

The results of the study are novel and consequently there are very few salivary-based manuscripts in the literature for comparison. Likewise, there are only a small number of manuscripts relating adiponectin and IGF-1 to breast cancer. With this in mind, the author will attempt to explain the findings.

The overall findings of this study coincide with Nigro et al. and Antonelli et al. concerning the presence of adiponectin and IGF-1 in saliva and its alteration in the presence of disease and exercise.^[29,30] In the manuscript of Nigro et al., they suggest that there were no significant differences be-

tween obese individuals and healthy controls with respect to adiponectin concentrations. This finding supports the results of this study. As shown in Table 1, there are no significant mean value differences of adiponectin between obese and healthy individuals. With respect to IGF-1 Antonelli et al., demonstrated the presence of IGF-1 in saliva and their concentrations coincide with the results of this study.^[30] Additionally, Nam et al. demonstrated that there were no differences in serum IGF-1 concentrations with respect to obesity.^[31] The Nam et al. study supports the findings presented in Table 1. Taken together, this suggests that these proteins are present in saliva and are not directly associated with obesity.

Table 1 illustrates an increased level of salivary adiponectin among the cancer cohort as compared to the healthy controls. This significance is in accordance with Karaduman et al.^[32] Karaduman et al. found higher tissue adiponectin concentrations among cancer tumors as compared to control tissues. This is contrary when compared to serum adiponectin levels, which are inversely related to breast cancer tissue levels.^[33-35] One possible explanation may be that adiponectin plays an important role in glucose metabolism in which a decrease in the serum adiponectin levels is correspond to an increase in the glucose levels. High glucose levels stimulate the proliferation of cultured breast cancer cells it can therefore be speculated that adiponectin modulates the breast cancer progression by affecting the glucose metabolism pathway.^[33-35] Taken together, this association might be partly explained by the low serum adiponectin levels seen in serum from obese breast cancer patients.^[33-35]

However, the secretory pathway of adiponectin is very complex, involving both the classical ER-Golgi pathway as well as unconventional secretory mechanisms such as an exosome-mediated pathway.^[36,37] One possible explanation for the salivary and cancer tissue adiponectin concentrations being elevated maybe due the presence adiponectin carrying exosomes. Adiponectin exists as a low-molecular-weight, middle-molecular-weight and high-molecular-weight forms in the circulation, with the middle-molecular-weight forms being the predominant form in the serum. High-molecular-weight forms are present principally in the exosome fraction.^[36,37] The exosomes when shed by the breast cancer tissue circulate and attach to the cellular membrane of the recipient or target cell. In this case, salivary gland tissue which is histo-physiologically similar to mammary tissue.^[25] Hence, the exosomes serve as an intercellular messenger which stimulate adiponectin secretion into saliva. Taken together, this suggests that exosome-associated adiponectin^[37] and exosome-free adiponectin may have different physiological and pathological functions in vivo and may elucidate the

elevated adiponectin levels in salivary and cancer tissues as compared to the lower levels in serum.^[26] If this explanation is correct, saliva may be a better media than serum for studying breast cancer progression as it reflects the true nature of neoplastic activity in a “real time” situation.^[25]

The summary for tumor stage, tumor size and nodal status are shown in Table 4 while Table 5 exhibits mean values for estrogen, progesterone and Her2/neu receptor status. These clinicopathological characteristics were not significant for either adiponectin or IGF-1. The adiponectin results agree with the findings of Karaduman et al.^[32] Interestingly, Miyoshi et al. reported that the low serum adiponectin levels were significantly associated with large tumor size (> 2 cm) and high histological grade (2 + 3), indicating that tumors with high proliferation activity are more likely to develop under the low adiponectin condition.^[33] As shown in Table 4 and Figure 1, this study tends to indicate that the opposite is true for salivary concentrations of adiponectin.^[33]

In conclusion, we have shown a significant association between the salivary adiponectin levels and the presence of breast cancer. Unfortunately, IGF-1 was not as indicative biomarker as adiponectin; however, IGF-1 did correlate with adiponectin concentrations implying that it may be playing a secondary role in breast cancer progression.^[38] As suggested by Maura et al., adiponectin activates multiple pathways and may modulate IGF-1 stimulatory effects in breast cancer cell via the IGF-1R.^[38]

One question emanating from the research is which media profile is correct? Are adiponectin concentrations elevated in both cancer tissue and saliva or are they are lowered as in serum? It is probably a combination of all three findings as all three probably are involved with differing molecular pathways. If this is the case, then the study of cancer progression may need to include other body fluids as well including saliva and urine along with serum/plasma and tissues.^[26]

These results seem to suggest a possibility that salivary adiponectin levels could be a risk factor for breast cancer and may provide a new insight into understanding of breast cancer risk. The limitation of the present study lies in that this is a case-control study with a small sample size. The preliminary results need to be confirmed by a prospective study with a larger number of subjects so that the data can be further stratified. Post-treatment and longitudinal data needed to be included to determine if the marker has any value as a prognostic indicator or a marker for tumor recurrence.

CONFLICTS OF INTEREST DISCLOSURE

The authors declare that there is no conflict of interest statement.

REFERENCES

- [1] Centers for Disease Control and Prevention. Summary health statistics for U.S. adults: National Health Interview Survey, 2010. Hyattsville, MD: National Center for Health Statistics. Vital & Health Statistics. 2012; 10(252).
- [2] Havel PJ. Control of energy homeostasis and insulin action by adipocyte hormones: leptin, acylation stimulating protein, and adiponectin. *Curr Opin Lipidol*. 2002; 13: 51-9. PMID:11790963 <https://doi.org/10.1097/00041433-200202000-00008>
- [3] Ryan AS, Berman DM, Nicklas BJ, et al. Plasma adiponectin and leptin levels, body composition, and glucose utilization in adult women with wide ranges of age and obesity. *Diabetes Care*. 2003; 26: 2383-8. PMID:12882866 <https://doi.org/10.2337/diacare.26.8.2383>
- [4] Gavrilu A, Chan JL, Yiannakouris N, et al. Serum adiponectin levels are inversely associated with overall and central fat distribution but are not directly regulated by acute fasting or leptin administration in humans: cross-sectional and interventional studies. *J Clin Endocrinol Metab*. 2003; 88(10): 4823-31. PMID:14557461 <https://doi.org/10.1210/jc.2003-030214>
- [5] Kang JH, Yu BY, Youn DS. Relationship of serum adiponectin and resistin levels with breast cancer risk. *J Korean Med Sci*. 2007; 22(1): 117-21. PMID:17297263 <https://doi.org/10.3346/jkms.2007.22.1.117>
- [6] Tworoger SS, Eliassen AH. Plasma adiponectin concentrations and risk of incident breast cancer. *J Clin Endocrinol Metab*. 2007 Jan 9. PMID:17213279 <https://doi.org/10.1210/jc.2006-1975>
- [7] Miyoshi Y, Funahashi T, Kihara S, et al. Association of serum adiponectin levels with breast cancer risk. *Clin Cancer Res*. 2003; 9(15): 5699-704. PMID:14654554
- [8] Bougoulia M. Effect of weight loss with or without Orlistat treatment in adipocytokines, inflammation, and oxidative markers in obese women. *Hormones*. 2006; 5(4): 256-9. <https://doi.org/10.14310/horm.2002.11190>
- [9] Rosen CJ, Pollak M. Circulating IGF-I: New Perspectives for a new century. *Trends Endocrinol Metab*. 1999; 10(4): 136-41. [https://doi.org/10.1016/S1043-2760\(98\)00126-X](https://doi.org/10.1016/S1043-2760(98)00126-X)
- [10] Voorhoeve PG, van Rossum EF. Association between an IGF-I gene polymorphism and body fatness: differences between generations. *Eur J Endocrinol*. 2006; 154(3): 379-88. PMID:16498050 <https://doi.org/10.1530/eje.1.02101>
- [11] Sweeney C, Murtaugh MA. Insulin-like growth factor pathway polymorphisms associated with body size in Hispanic and non-Hispanic white women. *Cancer Epidemiol Biomarkers Prev*. 2005; 14(7): 1802-9. PMID:16030120 <https://doi.org/10.1158/1055-9965.EP1-05-0149>
- [12] Smith SR. The endocrinology of obesity. *Endocrinol Metab Clin North Am*. 1996; 25: 921-42. [https://doi.org/10.1016/S0889-8529\(05\)70362-5](https://doi.org/10.1016/S0889-8529(05)70362-5)
- [13] Park MJ, Kim HS, Kang JH, et al. Serum levels of insulin-like growth factor (IGF)-I, free IGF-I, IGF binding (IGFBP)-1, IGFBP-3 and insulin in obese children. *J Ped Endocrinol Metabol*. 1999; 12: 139-44. <https://doi.org/10.1515/JPEM.1999.12.2.139>
- [14] Frystyk J, Vestbo E, Skjaerbaek C, et al. Free insulin-like growth factors in human obesity. *Metabolism*. 1995; 44: 10 suppl 4: 37-44. [https://doi.org/10.1016/0026-0495\(95\)90219-8](https://doi.org/10.1016/0026-0495(95)90219-8)
- [15] Ballerini MG, Ropelato MG, Domene HM, et al. Differential impact of simple childhood obesity on the components of the hormone-insulin-like growth factor (IGF)-IGF binding proteins axis. *J Ped Endocrinol Metab*. 2004; 17: 749-57. <https://doi.org/10.1515/JPEM.2004.17.5.749>
- [16] Attia N, Tamborlane WV, Heptulla R, et al. The metabolic syndrome and insulin-like growth factor I regulation in adult obesity. *J Clin Endocrinol Metab*. 1998; 83: 1467-71. PMID:9589640
- [17] Edén EB, Burman P, Holdstock C, et al. Effects of gastric bypass on the GH/IGF-I axis in severe obesity and a comparison with GH deficiency. *Eur J Endocrinol*. 2006; 154(1): 53-9. PMID:16381991 <https://doi.org/10.1530/eje.1.02069>
- [18] Frystyk J, Ledet T, Møller N, et al. Cardiovascular disease and IGF-1. *Circulation*. 2002; 106(8): 893-5. PMID:12186788 <https://doi.org/10.1161/01.CIR.0000030720.29247.9F>
- [19] Juul A, Scheike T, Davidsen M, et al. Low serum insulin-like growth factor 1 is associated with increased risk of ischemic heart disease: A population based case-control study. *Circulation*. 2002; 106(8): 939-44. PMID:12186797 <https://doi.org/10.1161/01.CIR.0000027563.44593.CC>
- [20] Hankinson SE. Circulating concentration of IGF-1 and risk of breast cancer. *Lancet*. 1998; 357: 1393-6. [https://doi.org/10.1016/S0140-6736\(97\)10384-1](https://doi.org/10.1016/S0140-6736(97)10384-1)
- [21] Morimoto LM, Newcomb PA. Insulin-like growth factor polymorphisms and colorectal cancer risk. *Cancer Epidemiol Biomarkers Prev*. 2005; 14(5): 1204-11. PMID:15894673 <https://doi.org/10.1158/1055-9965.EPI-04-0695>
- [22] Wei EK, Ma J. A prospective study of C-peptide, insulin-like growth factor-I, insulin-like growth factor binding protein-1, and the risk of colorectal cancer in women. *Cancer Epidemiol Biomarkers Prev*. 2005; 14(4): 850-5. PMID:15824155 <https://doi.org/10.1158/1055-9965.EPI-04-0661>
- [23] Lukanova A, Kaaks R. Endogenous hormones and ovarian cancer: epidemiology and current hypotheses. *Cancer Epidemiol Biomarkers Prev*. 2005; 14(1): 98-107. PMID:15668482
- [24] Irwin ML, McTiernan A. Relationship of obesity and physical activity with C-peptide, leptin, and insulin-like growth factors in breast cancer survivors. *Cancer Epidemiol Biomarkers Prev*. 2005; 14(12): 2881-8. PMID:16365005 <https://doi.org/10.1158/1055-9965.EPI-05-0185>
- [25] Streckfus C, Bigler L. A Catalogue of Altered Salivary Proteins Secondary to Invasive Ductal Carcinoma: A Novel in Vivo Paradigm to Assess Breast Cancer Progression. *Scientific Reports*. 2016; 30800(6): 1-18. <https://doi.org/10.1038/srep30800>
- [26] Streckfus CF, Bigler L, Dellinger TD, et al. The presence of c-erbB-2, and CA 15-3 in saliva and serum among women with breast carcinoma: A preliminary study. *Clin Cancer Res*. 2000; 6(6): 2363-70. PMID:10873088
- [27] Giuliano AE, Connolly JL, Edge SB, et al. Breast Cancer-Major changes in the American Joint Committee on Cancer eighth edition cancer staging manual. *CA Cancer J Clin*. 2017; 14: 1-14. <https://doi.org/10.3322/caac.21393>
- [28] Deng T, Lyon CJ, Bergin S, et al. Obesity, inflammation and cancer. *Ann Rev Pathol*. 2016; 11: 421-49. PMID:27193454 <https://doi.org/10.1146/annurev-pathol-012615-044359>
- [29] Nigro E, Piombino P, Scudiero O, et al. Evaluation of salivary adiponectin profile in obese patients. *Peptides*. 2015; 63: 150-5. PMID:25481860 <https://doi.org/10.1016/j.peptides.2014.11.007>
- [30] Antonelli G, Cappellin E, Gatti R, et al. Measurement of free IGF-I saliva levels: perspectives in the detection of GH/IGF axis in athletes. *Clin Biochem*. 2007; 40(8): 545-50. PMID:17321513 <https://doi.org/10.1016/j.clinbiochem.2007.01.014>
- [31] Nam SY, Lee EJ, Kim KR, et al. Effect of obesity on total and free insulin-like growth factor (IGF)-1, and their relationship to IGF-binding protein (BP)-1, IGFBP-2, IGFBP-3, insulin, and growth

- hormone. *Int J Obes Relat Metab Disord.* 1997; 21(5): 355-9. PMID:9152736 <https://doi.org/10.1038/sj.ijo.0800412>
- [32] Karaduman M, Bilici A, Ozet A, et al. Tissue levels of adiponectin in breast cancer patients. *Med Oncol.* 2007; 24(4): 361-6. PMID:17917082 <https://doi.org/10.1007/s12032-007-0021-0>
- [33] Miyoshi Y, Funahashi T, Kihara S, et al. Association of serum adiponectin levels with breast cancer risk. *Clin Cancer Res.* 2003; 9(15): 5699-704. PMID:14654554
- [34] Mantzoros C, Petridou E, Dessypris N, et al. Adiponectin and breast cancer risk. *J Clin Endocrinol Metab.* 2004; 89(3): 1102-7. PMID:15001594 <https://doi.org/10.1210/jc.2003-031804>
- [35] Chen DC, Chung YF, Yeh YT, et al. Serum adiponectin and leptin levels in Taiwanese breast cancer patients. *Cancer Lett.* 2006; 237(1): 109-14. PMID:16019138 <https://doi.org/10.1016/j.canlet.2005.05.047>
- [36] Phoonsawat W, Aoki-Yoshida A, Tsuruta T, et al. Adiponectin is partially associated with exosomes in mouse serum. *Biochem Biophys Res Commun.* 2014; 448(3): 261-6. PMID:24792183 <https://doi.org/10.1016/j.bbrc.2014.04.114>
- [37] DeClercq V, d'Eon B, McLeod RS. Fatty acids increase adiponectin secretion through both classical and exosome pathways. *Biochim Biophys Acta.* 2015; 1851(9): 1123-33. PMID:25900100 <https://doi.org/10.1016/j.bbailip.2015.04.005>
- [38] Mauro L, Naimo GD, Ricchio E, et al. Cross-talk between adiponectin and IGF-IR in breast cancer. *Front Oncol.* 2015; 5: 157. PMID:26236690 <https://doi.org/10.3389/fonc.2015.00157>