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Assessment of Insulin and Cortisol Levels in Iraqi Women with Breast Cancer

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ABSTRACT

Hormones are thought to primarily influence cancer risk by regulating the pace of cell division, cell differentiation, and the amount of cancer-vulnerable cells. Higher insulin levels and cortisol have been linked to some cancers, including breast cancer. Here, we evaluated the relationship between insulin and cortisol signaling and cancer, as well as the effect of these two hormones on organs, subcellular elements, and the process of carcinogenesis. We also consider the time points to prevent and cure breast cancer in insulin signaling. The ultimate objective is to enhance general health. Insulin and cortisol levels were tested and the results showed much higher insulin levels in breast cancer patients compared with control ($p<0.001$), whereas cortisol levels tested were lower in breast cancer patients compared with control with no statistically significant changes ($p>0.05$).

KEYWORDS: Breast cancer; insulin, cortisol; metabolic health.

الخلاصة

يُعتقد أن الهرمونات تؤثر بشكل أساسي على مخاطر الإصابة بالسرطان من خلال تنظيم وتيرة انقسام الخلايا وتمايز الخلايا وكمية الخلايا المعرضة للسرطان، وقد تم ربط مستويات الأنسولين المرتفعة والكورتيزول ببعض أنواع السرطان، بما في ذلك سرطان الثدي. هنا، نقوم بتقييم العلاقة بين إشارات الأنسولين والكورتيزول والسرطان، وكذلك تأثير الأنسولين والكورتيزول على الأعضاء والعناصر تحت الخلوية وعمليات السرطان. نحن أيضًا نأخذ في نظر الاعتبار النقاط الزمنية للوقاية من سرطان الثدي وعلاجه في إشارات الأنسولين. الهدف النهائي هو تعزيز الصحة العامة. تم اختبار مستويات الأنسولين والكورتيزول وأظهرت النتائج ارتفاعاً كبيراً في مستويات الأنسولين لدى مرضى سرطان الثدي مقارنةً بمجموعة التحكم ($p<0.001$)، بينما كانت مستويات الكورتيزول المقاسة أقل لدى مرضى سرطان الثدي مقارنةً مع مجموعة التحكم مع عدم وجود تغييرات ذات دلالة إحصائية ($p>0.05$).

INTRODUCTION

Women's risk of developing breast cancer is influenced by a variety of variables, including age, breast gland mass, and hormonal stability. Age is the most significant and determining factor, with 32% of women being over 60 [1]. By altering the mammary gland's growth, hormonal factors, such as early menstruation and later amenorrhea, raise the risk of breast cancer. Breastfeeding lowers the risk of developing breast cancer and protects against its later occurrence. The risk of breast cancer decreases with overall nursing duration [2]. A number of research have tried to figure out whether there is a connection between BMI and cancer. Since these studies' findings varied, it can be inferred that there is a complex relationship between BMI and cancer risk, possible breast cancer risk, and general metabolic health [3].

Insulin is a potent stimulator of various biological pathways that result in aggressive breast cancer, making it a crucial factor in the development of type 2 diabetes, cancer, and the process of cell carcinogenesis [4]. Insulin is a cell survival factor (anti-apoptosis) that promotes cancer cell migration and increases their capacity to invade. Additionally, it promotes the growth of several human breast cancer cell lineages via processes that make use of the Akt and phosphatidylinositol-3 kinase signaling pathways. Breast cancer cells are impacted by hyperinsulinemia via the endocrine system [5].

Both cancer and hyperinsulinemia are extremely common pathophysiological diseases linked to

significant morbidity and mortality globally. Both breast cancer cells and healthy mammary gland tissue respond well to insulin as a key mitogen. Insulin is a direct mitogen (stimulates cell division, mitosis) and cell survival factor in breast cancer cells. The signaling pathways, insulin, and its receptor. Although insulin largely controls how carbohydrates, lipids, and proteins are metabolized, it also functions as a critical growth factor [6]. It encourages cell proliferation and migration while simultaneously blocking apoptosis; these actions may be exacerbated by the development of insulin resistance and the resultant impairment of insulin-regulated metabolic pathways [7].

It has been suggested that psychological stress exposure and stress-related cortisol release are linked to an increased risk of breast cancer. It interacts with specific brain regions to regulate mood and arouse fear [8]. The body's functions that involve cortisol are significant in many ways. When a person is running, or engaged in "fight or flight," cortisol has a widespread influence throughout the body and is produced in huge levels to aid in response and stress management [9]. Numerous clinical research have shown this connection between cortisol levels and cancer severity, as well as an increase in breast cancer mortality and recurrence [10]. Such abnormalities have been theorized to be caused by a variety of factors, including a flaw in the hypothalamus-pituitary-adrenal (HPA) feedback system, heightened susceptibility to stress, an inability to deactivate cortisol, and even irregular sleep patterns [11]. Stress and mammary gland apoptosis In vitro studies have shown that cortisol inhibits apoptosis and necrosis in the mammary glands and stimulates the zodiac pathways in non-cancerous epithelial cells [12]. both of which are consistent with its role in promoting lactation. According to studies, psychological stress and repeated stress may promote the growth of tumors by inhibiting the clearance of genetically modified cells [13],[14].

MATERIALS AND METHODS

The Solid Phase Enzyme-Linked Immunosorbent Assay serves as the foundation for the Insulin (Human) CLIA Kit. One anti-Insulin antibody was used in the antibody-enzyme (horseradish peroxidase) conjugate solution, and a second anti-Insulin antibody was used in the solid phase inhibitory assay method (microtiter wells).

The Procedure

For insulin:

1. 50 µl l of insulin standards, samples, and controls were added to the appropriate wells after the required number of coated wells were placed in the holder. The mixture was then gently mixed well for 10 seconds.
2. To each well, we added 100 µl of the enzyme conjugate reagent before mixing for 30 seconds. In this step, it's crucial to have the mixing finished. 60 minutes of room temperature incubation is required.
3. By pouring the contents of the plate into the waste container, we eliminated the vacuum mixture. The plate was then cleaned and emptied five times with a buffer wash solution (300 l in each well), and any leftover water was then wiped off with absorbent paper.
4. Each well received 100 µl of the Chemiluminescence substrate reagent. We gently combined for 10 seconds. For 20 minutes, samples were incubated at room temperature in the dark.
5. After adding 100 µl of Stop Solution to each well to stop the reaction, we gently mixed for 10 seconds until the blue color turned entirely yellow.
6. Within 15 minutes, we used a microtiter plate reader to read the optical density at 450 nm.

We used a pure cortisol antigen to label ABEI and an anti-cortisol monoclonal antibody to label FITC in the competitive immunoluminometric test for cortisol. We thoroughly combined the sample, the calibrator or control, the FITC label, the ABEI label, and the magnetic microbeads coated with anti-FITC. We then incubated the mixture at 37 °C to form antibody-antigen complexes, sedimented them in a magnetic field, and poured the supernatant before performing a single cycle of washing. A flash chemiluminescent reaction was then started after the addition of the chemicals. Within 3 seconds, a photomultiplier measures the light signal as RLU, which is proportional to the amount of cortisol present in the samples.

Procedure Table

Sample, calibrator **ABEI Label** **FITC Label** **Nano magnetic microbeads**	40µl +80µl +80µl +20µl
Incubation	15 min
Cycle washing	400µl
Measurement	3 s

RESULTS AND DISCUSSION

The mean±SD of insulin concentration in BC patients and healthy control individuals was 19.43±15.79 µg/dl and 10.21±5.92 µg/dl, respectively, Table 1 and Figure 1. The results showed that, insulin levels increased significantly **(**p<0.001**)** in patients compared to the control group.

Table 1. Levels of insulin and Cortisol for patients and control groups.

Parameters	Mean level ± SD of groups		Area under curve	Probability
	Patients	Control		
Cortisol (µg/dl)	12.66 ±8.63	10.64 ±6.13	0.553	p > 0.05
Insulin (µl unit/ml)	19.43±15.79	10.21±5.92	0.710	p < 0.001

* Significant at (p <0.01), Non-Significant at (p>0.05).

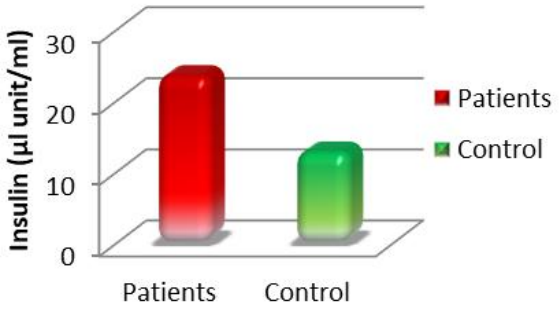

Figure 1. Diagram shows levels of insulin for patients and control groups.

The results of this study are in line with earlier research[15],[16] that showed significantly higher insulin levels in BC patients than in control volunteers.

The insulin receptor is frequently overexpressed in human breast tumors, and this overexpression can change the phenotypic of human breast mammary cells [17]. Insulin is a major factor in both type-2 diabetes and cancer. Insulin is a powerful hormone that opens up numerous pathways that fuel the biology of aggressive breast cancer The major regulators of cellular homeostasis are activated by the binding of insulin-to-insulin receptors in cells. The majority of aggressive human malignancies exhibit physiologic dysregulation of these signaling pathways [4]. Given the overexpression of the insulin receptor (IR), the role of insulin in breast cancer outcomes is biologically plausible [19], Recent research has revealed that women with insulin-receptor-positive breast cancer have a worse prognosis for tumor development than women with insulin-receptor-negative breast cancer [20].

We expected that cancer patients would demonstrate greater insulin resistance with increased BMI in comparison to non-cancerous control groups given the increased risk of diabetes in patients with carcinoid tumors and the potential synergistic effects of malignancy and obesity. We looked at the interaction between cancer status and BMI as predictors of overall insulin sensitivity to test this theory. Our bodies are instructed to store extra sugar when there is a lot of insulin and blood sugar in our blood stream. Although our muscles and liver can store some sugar, when these are full, our body begins to store the extra sugar as fat. Naturally, this leads to weight growth. According to recent research, having a high body mass index increases insulin resistance and decreases insulin sensitivity, which all help to increase the risk of developing breast cancer [21].

The mean±SD of cortisol concentration in BC patients and healthy control individuals was 12.66±8.63 µg/dl and 10.64±6.13 µg/dl, respectively, Table 1 and Figure 2 The statistical analysis showed that even the cortisol concentration of patients is higher than control but statistically these differences are not significant (p>0.05).

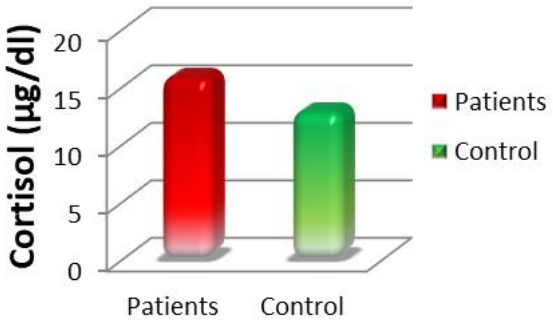

Figure 2. Diagram shows levels of cortisol for patients and control groups.

These results are in agreement with other studies[22],[23] that presented an increases in the level of cortisol in BC patients than in control subjects.

Acute and chronic stress have both been investigated in relation to breast cancer relapse, with the majority of research demonstrating a stronger link between chronic stress and breast cancer than between acute and acute stress [24].

The measurement of cortisol levels is one of the most reliable ways to gauge someone's physiological stress [25]. More than 60% of breast cancer patients had aberrant cortisol levels throughout the day, and their mortality rate was much greater than that of women with normal cortisol levels [26]. Such abnormalities could be caused by a variety of factors, including a malfunction in the hypothalamic-pituitary-adrenal (HPA) feedback system, heightened susceptibility to stress, an inability to deactivate cortisol, and even sleep disturbances.

CONCLUSIONS

High levels of insulin resistance in postmenopausal women are linked to a higher incidence of breast cancer and a higher rate of all-cause mortality after breast cancer because insulin dramatically promotes cell proliferation. A study of the insulin hormone level in BC affected women showed a significant increase in the hormone level. It is important to realize that IR might affect both the risk and the diagnosis of BC. In order to do this, it is necessary to be aware of such associations from the outset and to offer recommendations, such as lifestyle changes and/or IR adjustments, while providing routine screening services, not only in developing countries but also in developing and low-income countries. Since there was a minor increase in cortisol in the sick group compared to the control group, it is important that we concentrate our research efforts in areas that would logically and quickly progress the discipline. For instance, based on conceptual models of etiology and characteristics of the assessment measure, the best biological and psychometric measures of psychosocial distress have not been discovered and used for community-based investigations of stress and breast cancer risk. By using the proper batteries for measurements and simulations, as well as in large-scale experiments and stress observational studies, this transformation can be achieved.

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