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Original Article

A Comparative Study to Determine LDH Enzyme Levels in Serum Samples of Women with Breast Cancer and Women with Breast Cancer and Type 2 Diabetes Mellitus

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ABSTRACT

Background: Cancer and diabetes both have been linked to aberrant lactate metabolism, and a high level of lactate formation is a prominent biological feature of both diseases. Among glycolysis enzymes, lactate dehydrogenase (LDH) is an exciting possible pharmacological target for the cancer treatment. Drawing a comparison between diabetes and cancer, the importance of the excessive lactate generation in the diabetes-cancer interaction should not be overlooked. Therefore, this research was designed to determine the connection between lactic dehydrogenase enzyme (LDH) and the blood glucose in serum samples of women with breast cancer-associated to those with breast cancer and type 2 diabetes mellitus (T2DM).
Samples and methods: Seventy-five samples were categorized into three equal groups; group 1: Non-breast cancer or diabetes (control subjects C), group 2: patients suffering from breast cancer (BC), and group 3: patients suffering from breast cancer (BC) and type 2 diabetes mellitus (BC and T2DM), in all samples, the amounts of the total LDH and glucose were measured.
Results: The LDH level increased significantly ($p \leq 0.001$) in (BC and T2DM) group and (BC) group in comparison with the control group (C), respectively, and a significant increase in LDH ($p \leq 0.01$) was obtained in (BC and T2DM) group versus the (BC) group. In addition, compared with the control group, the results indicated that the BC and BC and T2DM groups had the higher levels of glucose and LDH.
Conclusion: The LDH is a useful diagnostic marker for the metabolic syndrome and can be used to investigate the metabolic changes linked to breast cancer and diabetic complications. Many years ago, it was known that increased LDH is a poor predictor of cancer outcome. LDH may have a prognostic effect on breast cancer metastases, but this is unclear. Concerning the results of this investigation, we speculate that LDH could act as a predictor of breast cancer. Increasingly, the data suggest that diabetes mellitus is linked to an increased risk of cancer and a significant cause of death in cancer patients.

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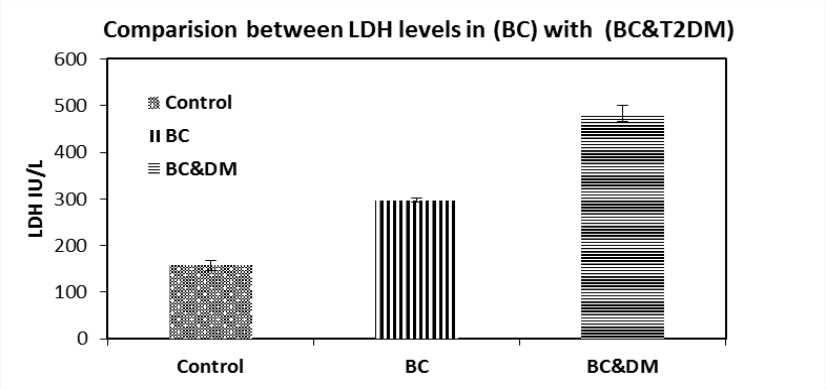
GRAPHICAL ABSTRACT



Introduction

Hyperglycemia is a symptom of diabetes mellitus (DM), a chronic endocrine condition caused by insufficient pancreatic insulin secretion, insufficient insulin sensitivity throughout the body, or both [1, 2]. Around 200 million people have diabetes as of 2010, and the World Health Organization (WHO) predicts that the number will rise to around 300 million by 2025 [3]. As a result of the pathophysiology complexity associated with diabetes, both environmental and genetic factors may be relevant in this situation [4].

According to the scientific research, diabetes is associated with a higher incidence, faster progression, and greater aggressiveness of several malignancies. Among the numerous cancer forms, research has demonstrated a link between diabetes and the chance of developing breast cancer [5]. Although the higher risk of cancer in diabetics may only be minor to moderate, given that diabetes is a worldwide epidemic [6].

The metabolic disorder known as type 2 diabetes mellitus (T2DM), characterized by hyperglycemia, is caused by the diminished insulin action and insulin synthesis in the pancreatic beta cells [7]. According to the recent studies, type two diabetes is a distinct risk factor for the progression of different cancers [8, 9]. In addition, cancers that are related to one type of diabetes may not be linked to the other, such as breast cancer, which is mainly linked to type 2 diabetes and not type 1 diabetes [10].

The most common type of malignancy in women is breast cancer (23 percent of all malignancies) [11], and it ranks second overall when both sexes are taken into account [12]. Several biochemical indicators have been tested to determine malignancy for the early diagnosis of carcinomas of various origins [13].

The association between cancer cell metabolism and metastatic potential has recently attracted attention because it has been discovered that cancer cells use glycolysis or the oxidative phosphorylation more frequently depending on diverse intrinsic or extrinsic variables [14].

In mammalian cells, two key metabolism routes for creating ATP are glycolysis and oxidative phosphorylation [15]; even though glycolysis does not produce as much ATP per mole of glucose as the oxidative phosphorylation does; the frequent doubts remain about the efficacy of both mechanisms in supporting cancer cell proliferation [16].Notably, the association between aerobic glycolysis and cancer is often regarded as a useful metabolic foundation for the creation of innovative anticancer treatments. Among glycolysis enzymes, lactate dehydrogenase (LDH) is an exciting possible target for cancer treatment. However more significantly, the link between aerobic glycolysis and cancer is widely regarded as a functional biochemical basis for developing new strategies to fight cancer [17]. Lactate, previously thought to be a waste product of glycolysis, is now understood to have a crucial role in the regulation of insulin resistance, diabetes mellitus, growth, development, and spreading of cancer [18].

An essential stage in the creation of cell energy is the transformation of lactate to pyruvate, which

is catalyzed by the enzyme LDH [19]. Furthermore, the end of the glycolysis process is considered the most interesting molecular target for developing new molecular targets, such as glycolysis inhibitors for the potential cancer treatment [20].

Different serum biochemical indicators were employed for breast cancer. However, LDH is the easiest and the least expensive to assess [21]. In primary breast cancers, LDH inhibition has an anti-proliferative impact [22]. Deregulated LDH levels have been previously observed in different cancers, including endometrial pancreatic, breast, nasopharyngeal, gastric, and bladder [23].

Materials and Methods

Samples

The Ethical Committee at the Al-Amal National Cancer Hospital in Baghdad, Iraq, accepted the collection of samples for this study. The participants answered a health history questionnaire created by the researchers. Some demographic factors were examined, counting age in years, and previous experience with diabetes. To find subjects for this inquiry, the following criteria were created.

- 39-55 years old.
- A glucose level of less than 200 mg/dl for diabetics and a fasting glucose range of 80 to 110 mg/dl for those in the clinical range.
- LDH 100-240 IU/L for the normal category

Table 1 summarizes the key features of the participants. The analysis's 75 individuals were divided into three equal groups, with group 1 being a control group of healthy women (Control), in the age range of 39 to 50 years old; group 2 being a group of samples with breast cancer (BC), in the age range of 39 to 55 years old; and group 3 being a group of samples with breast cancer and type 2 diabetes mellitus (BC and T2DM), in the age range of 42 to 53 years old.

Sample collection

After 10-12 hours overnight fast, each participant had 5 mL of blood taken from the antecubital vein and placed in a sterile Eppendorf container. The serum was separated after 10 minutes of centrifuging the sample at 2000 rpm. The tubes were kept at room temperature for about 30 minutes to facilitate blood coagulation. The serum was kept at -20 °C until it was examined. The estimation of LDH and glucose levels was done by using this serum sample.

Biochemical analysis

Assessment of serum glucose level and total lactate dehydrogenase activity

The serum samples were used for the estimation of LDH and glucose. The LDH levels in serum samples were determined by using a commercially available kit from Roche Diagnostics (Roche Elecsys modular Cobas e 411 (electrochemiluminescence immunological assay)) (Manheim, Germany). Vassault's principles were used for its determination. A spectrophotometer set at 340 nm was used to assess NADH consumption [24]. CHEMELEX, S.A. used a quantitative approach to assess blood glucose in serum samples.

Statistical Analyses

Data analysis was carried out by using Microsoft Excel 2016. The mean and standard deviation (SD) are descriptive statistics for the continuous data. A t-test is used to compare matched sample sets to determine statistical significance.

Results and Discussion

The LDH level increased significantly in both (BC and T2DM) group and (BC) group compared with the control group (C), respectively, and a significant increase in LDH was obtained in the (BC and T2DM) group versus (BC) group, as displayed in Figure 1. Table 1 summarizes the key features of the participants.

Furthermore, the results showed an elevation in the glucose and LDH levels of the BC, and BC and T2DM groups compared with the control group, as indicated in Figure 2. The mean value average of LDH (IU/L) was observed in (BC and BC and T2DM) compared with control, BC= Breast cancer, BC and T2DM = Breast cancer with diabetes mellitus type 2).

The vital role that lactate dehydrogenase (LDH) plays in anaerobic metabolic pathways makes it

an enzyme of particular interest. In the present investigation, LDH levels did not significantly differ between the diabetic and BC groups and the control patients, which is consistent with findings from earlier studies [25]. Also, no significant change appeared in BC compared with BC and T2DM patients. The other studies concur with this study in demonstrating that LDH is unaffected by mild diabetes mellitus but raises the plasma levels of LDH in severe diabetes mellitus [26]. An enhanced rate of cell growth, migration, or invasions compared with normal cells may be the cause of the elevated LDH activity in cancer cells. In other words, compared with a normal cell population, a big cancer cell population requires a greater and faster energy supply. The LDH activity is an alternative used by cancer cells to meet this massive and urgent required energy, which is useful for metabolic needs and the aerobic glycolysis of malignant cells [27].

Table 1: The important characteristics (Age, LDH, and glucose) in different groups

Characteristic	G1 (control)	G2 B.C	G3 B.C & T2DM
Age (years old)	39-50	39-55	42-53
Mean LDH IU/L LDH (100-240)	158.2 ±10.68	4.03±296.6	472.16 15±8
Significant p (value) ***=p≤0.001 ** = p≤0.01 N= non-significant	N	**	***
Fasting Glucose (mg/dl) Normal range (70-110)	80.7±2.73	110.97±22.4	193.4±10.5

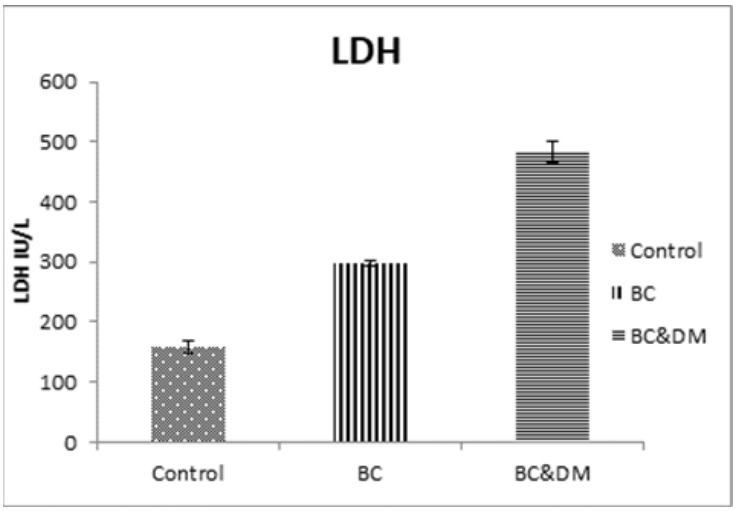


Figure 1: Determination of LDH level in different subjected species

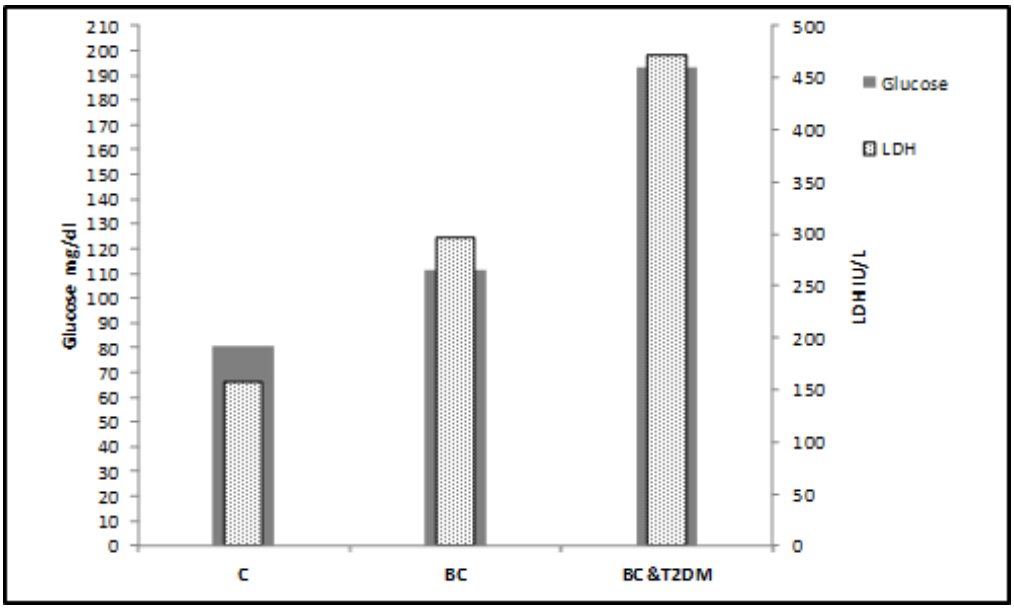


Figure 2: Forest plots of thiamine, ascorbic acid, and a combination of both on incidence rates during the intervention

The overexpression of the LDH gene in breast cancer cells compared with normal cells may be one of the possible explanations for the high LDH activity in cancer cells, the anaerobic glycolysis, and metabolism of these cancerous cells are both aided by the elevated LDH level [28].

On the other hand, the elevated lactate accelerates the onset and progression of diabetes and cancer by enhancing insulin resistance and the malignant phenotype of cancer cells [29].

More data suggests that diabetes mellitus is linked to an increased risk of cancer and a significant cause of death in cancer patients [30]. Diabetic patients with colorectal, breast, and endometrial cancers seem to have a much higher risk of dying from cancer than healthy people [31]. Numerous studies evaluated the predictive value of pre-existing DM in individuals with lung cancer, according to these studies patients with DM lived shorter lives than those without the condition [32].

The previous studies showed that in breast cancer tissue, lactate dehydrogenase A (LDH-A) was found to be expressed at the higher levels and was linked to invasiveness, serving as a marker for tumor progression [33]. Breast cancer cells express more genes and produce more LDH than nearby normal cells (in the pyruvate-reducing direction). In addition, clinical malignancies' overexpression of the LDH-A gene is frequently linked to disease progression and a poor prognosis [34].

Science has yet to discover a particular strategy to diagnose human breast cancer. Numerous attempts to find the best and most accurate prognostic prediction biomarkers for breast cancer [35]. An increased blood LDH level may reflect dynamic metabolic changes [36, 37].

Conclusion

Lactate metabolism metabolic pathways are crucial for understanding the physiological response and the development of common diseases like diabetes and cancer. In breast cancer patients, an increase in serum LDH values suggests a poor prognosis, surgical result, or the extensive metastases. In women with breast cancer who are getting treatment, the serum LDH levels can be monitored as a prognostic indicator. The increased blood LDH levels in these patients could be an early warning indicator of recurrence or metastasis.

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Authors' contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

We have no conflicts of interest to disclose.

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