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RESEARCH ARTICLE

Evaluating Serum Biomarkers (GLP-1r, AMPK, and Caspase-3) Levels for Early Detection of Diabetic Breast Cancer

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ABSTRACT

Breast tumours are the second most common cancer in women. A mammary cancer is a multi-step process that involves several cell types and is yet difficult to prevent globally. One of the best ways to prevent breast cancers is by early detection. In many affluent nations, the 5-year proportional persistence ratio for people with mammary tumours is over 80% because to early detection. Over the past ten years, significant progress has been made in both the understanding of mammary tumours and the development of preventative interventions. Diabetes, often known as diabetes mellitus (DM), is a collection of illnesses characterised by elevated blood sugar levels brought on by flaws in the body's capacity to make and/or utilize insulin. To evaluate serum GLP-1r, AMPK, and Caspase-3 levels in people with diabetes and breast cancer. The samples were taken from individuals at Al-Najaf Teaching Hospital and the Najaf Cancer Centre among 30/10/2023 and 1/3/2024. The blood samples were 120 blood samples acquired from Iraqi subjects. The participants included four groups and each group consist of 30 participants, G1 as control group, G2 as diabetic breast cancer patient group, G3 as diabetes type 2 patient group and G4 as breast cancer patient group. The results showed significantly lower levels of GLP-1r, AMPK, and Caspase-3 in the patient groups compared to the healthy control group. Low levels of GLP-1 receptor, AMPK, and Caspase-3 could serve as potential screening markers for early detection of diabetic breast cancer. These findings suggest these biomarkers may have clinical utility for improving early diagnosis and management of this condition.

INTRODUCTION

Mammary cancer is one of the most predominant human neoplasms(Anstey et al., 2017). Breast cancer develops when malignant cells proliferate. When cancerous cells form in the lining of the breast's milk-producing glands or ducts the condition is categorized as cancer. (Takahashi et al., 2020). Mammary cancer is the most common cancer in the world that murders and affects women (Ali et al., 2018). Currently, after cardiovascular diseases, breast neoplasm is the second largest cause of death for females in Iraq(Al-Abassi et al., 2018). However, according to the Iraqi Cancer Council, the percentage of women with breast malignancy is 34.06% greater than that of other cancer types(Pandey et al., 2021). Age, menstruation, alcohol use, hormonal factors, inadequate nutrition, environmental factors, hereditary factors, obesity, and other factors all increase the risk of developing breast cancer(Kamińska et al., 2015). At the nipple's peak, each lobe empties into a lactiferous duct. Near each lactiferous duct's end is a dilatation known as the lactiferous sinus. There

is columnar epithelium in the smaller ducts. The cell-based epithelium is found in two or three layers in larger ducts. The lining gets squamous and stratified towards their nipple apertures. The anatomy of the glandular components of the mammary gland undergoes significant modifications during life(Moore et al., 2021). Diabetes, also referred to as diabetes mellitus (DM), is a group of disorders marked by high blood sugar levels caused by deficiencies in the body's capacity to produce and/or use insulin. This illness is predominantly recognized by the degree of hyperglycemia that raises the risk of microvascular injury (retinopathy, nephropathy, and neuropathy) (Banday et al., 2020). It is related to a reduced life expectancy, significant injury from certain diabetes-related microvascular illnesses, an increased risk of macrovascular consequences and a worse quality of life (L. Wang et al., 2017). There are several pathogenetic mechanisms that result in the development of diabetes. These include operations that destroy beta cells in the pancreas, which results in insufficient insulin, and others that make the body resistant to the effects of insulin(Burrack et al., 2017). Because of variations in insulin sensitivity or lack thereof, insulin's insufficient effect on target tissues results in abnormalities in the metabolism of carbs, lipids, and proteins (Czech, 2017). A novel strategy to treating diabetes, glucagon-like peptide-1 (GLP-1) receptor agonists have favorable effects on weight, blood pressure, cholesterol, and beta-cell function in addition to improving glucose regulation (Prasad-Reddy et al., 2015). They have an impact similar to that of the incretin hormone GLP-1, which is secreted by the gut in reaction to eating. They have the following effects: they increase satiety, decrease glucagon release, slow gastric emptying, and increase insulin secretion. GLP-1 receptor agonists are a novel and useful way to enhance blood glucose regulation (Aroda, 2018). Weekly formulations could potentially enhance patient compliance. All things considered, they are useful medications for type 2 diabetics who are either unable to control their diabetes with metformin or who are intolerant to it (Trujillo et al., 2021). In eukaryotes, AMPK serves as the main energy sensor and regulator of energy homeostasis (Yuan et al., 2013). After activation, AMPK phosphorylates downstream targets to influence transcription and translation factors, growth and proliferation pathways, rate-limiting metabolic enzymes, and epigenetic regulators either directly or indirectly. Taken together, these lead to an increase in oxidative phosphorylation, autophagy, glucose uptake and metabolism, and a decrease in the production of proteins, fatty acids, cholesterol, and ribosomal RNAs (rRNAs), as well as a reduction in cell growth and proliferation (Garcia et al., 2017). A viable pharmaceutical target, particularly for the treatment of type 2 diabetes, AMPK is dysregulated in diabetes, obesity, cardiometabolic illness, and cancer because of its fundamental roles in metabolism (Guigas et al., 2016). Recent research has focused a lot of attention on the cysteine-aspartic acid protease Caspase-3, which plays amazing functions in tissue differentiation, regeneration, and neurological development (Asadi et al., 2022).

MATERIALS AND METHODS

Subjects

The study included 120 women participants, who were divided into four groups which are the experimental group consisted of 30 breast cancer patients with diabetic type2 and diabetic type2 of 30 and breast cancer of 30 and the control group consisting of 30 healthy individuals**.** The study contained the gathering of blood models as well as experimental methodologies permitted by the Al-Seder Medical City and Diabetes and Endocrinology Center and AL-furat Al-Awsat Teach Hospital and Oncology Hospital in Najaf Governorate. All research participants consented to the University of Al-Qadisiyah prior to the collection of samples. Additionally, from 30/10/2023 to 1/3/2024, all methods and procedures were followed in compliance with the rules and norms set forth by the Ethical Committee of the College of Medicine, University of Al-Qadisiyah.

Blood Sample Collection

Five milliliters of blood for respectively participant were together from vein puncture in sterile gel tubes and allowed to coagulate for a few minutes at room temperature, followed by separation of the

serum from the coagulate by centrifugation for 10 minutes at a 2012 x g. Then they were divided into several Eppendorf tubes and immediately frozen at -80 ℃ until used in the ELISA test.

Inclusion Criteria

Patients with diabetes mellitus and breast cancer, including those who had recently been diagnosed and those who had the disease spread to other parts of their bodies, should not undergo mastectomy.

Exclusion Criteria

Patients, who underwent a mastectomy, had ovarian cancer, pancreatic cancer, colorectal cancer, or inflammatory bowel disease was excluded from the study.

RESULTS

Demographic characteristics of control subjects and individuals with diabetes, breast cancer, and diabetic breast cancer

Table 1: Comparison of mean age between control subjects and individuals with diabetes, breast cancer, and diabetic breast cancer

Characteristic	Diabetes *n* = 30	Breast Cancer *n* = 30	Diabetic breast cancer *n* = 30	Control *n* = 30	*p*
Age (years)					
Mean ±SD	50.4 ± 7.9 A	47.6 ± 6.8 A	52.4 ± 6.2 A	51.6 ± 7.7 A	.06 O NS
Range	39 – 63	38 – 62	42 – 64	38 – 63	
BMI(kg/m^2)					
Mean ±SD	26.5 ± 2.35 A	28.04 ± 3.8 AB	26.9 ± 3.3 AC	25.6 ± 2.2 A	.0256 O*
Range	22.8 – 33.1	22.6 – 39.9	22 – 35	22 – 29.7	

n: number of cases; **SD**: standard deviation; **BMI**: Body Mass Index; **O**: one-way ANOVA; NS: not significant;* : significant at p ≤ 0.05. Capital letters **A**, **B** and **C** were used to indicate the level of significance following performance post hoc **LSD** test so that similar letters indicate no significant difference**,** whereas the different letters indicate significant difference.

Comparison of mean values of hematological characteristics among control subjects and individuals with diabetes, breast malignancy, and diabetic breast cancer

Table 2: Comparison of mean values of hematological characteristics control subjects and individuals with diabetes, breast malignancy, and diabetic breast malignancy

Characteristic	Diabetes *n* = 30	Breast cancer *n* = 30	Diabetic breast cancer *n* = 30	Control *n* = 30	*p*
WBC X10^9/L					
Mean ±SD	8.0 ± 2.1 A	6.1 ± 2.7 B	5.0 ± 2.2 B	6.5 ± 1.2 C	<0.001 O***
Range	3.8 – 14.8	1.3 – 13.1	1.8 – 11.2	4.1 – 9.2	
Haemoglobin (g/dl)					
Mean ±SD	11.4 ± 1.3 A	11.2 ± 1.2 A	11.3 ± 1.3 A	11.5 ± 0.8 A	0.7 O NS
Range	9.2 – 14.1	9.1 – 14.2	8.3 – 13.4	10 – 13	

n: number of cases; **SD**: standard deviation; **WBC**: white blood cells; **O**: one-way ANOVA; **NS**: not significant; *: significant at p ≤ 0.05; ***: noteworthy at p ≤ 0.001. Capital letters **A**, **B** and **C** were used to indicate the level of impact following performance post hoc **LSD** test so that similar letters indicate no significant difference**,** whereas the different letters indicate noteworthy difference.

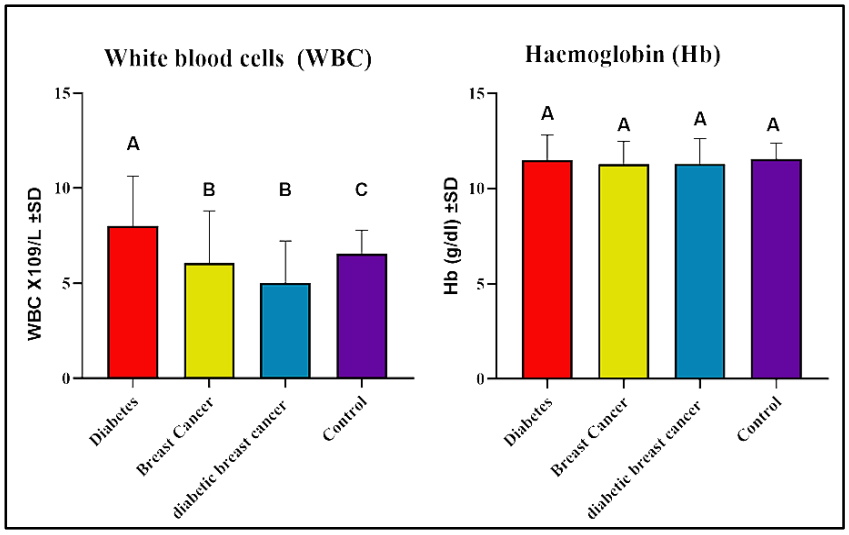

Figure 1: A depiction of the average of control group levels for hematological characteristics (White blood cell (WBC) and Haemoglobin (Hb) compared with diabetes, breast cancer, and diabetic breast cancer patients.

Comparison of blood urea and serum creatinine between individuals with diabetes, breast malignancy, diabetic breast cancer and control group

Table 3: Comparison of blood urea and serum creatinine between individuals with diabetes, breast malignancy, and diabetic breast cancer and control group

Characteristic	Diabetes *n* = 30	Breast cancer *n* = 30	Diabetic breast cancer *n* = 30	Control *n* = 30	*p*
Blood Urea (mg/dl)					
Mean ±SD	34.8 ± 12.1 A	31.3 ± 10.8 A	30.6 ± 12.3 A	27.1 ± 5.8 A	0.06 O NS
Range	18 – 58	18 – 62	15 – 59	16 – 38	
Serum Creatinine (mg/dl)					
Mean ±SD	0.73 ± 0.22 A	0.67 ± 0.25 A	0.74 ± 0.28 A	0.61 ± 0.16 A	0.4 O NS
Range	0.3 – 2.1	0.3 – 1.88	0.4 – 1.8	0.4 – 0.9	

n: number of cases; **SD**: standard deviation; **O**: one-way ANOVA; **NS**: not significant; *: significant at p ≤ 0.05; ***: noteworthy at p ≤ 0.001. Capital letters **A**, **B** and **C** were used to indicate the level of impact following performance post hoc **LSD** test so that similar letters indicate no significant alteration**,** whereas the different letters indicate noteworthy difference.

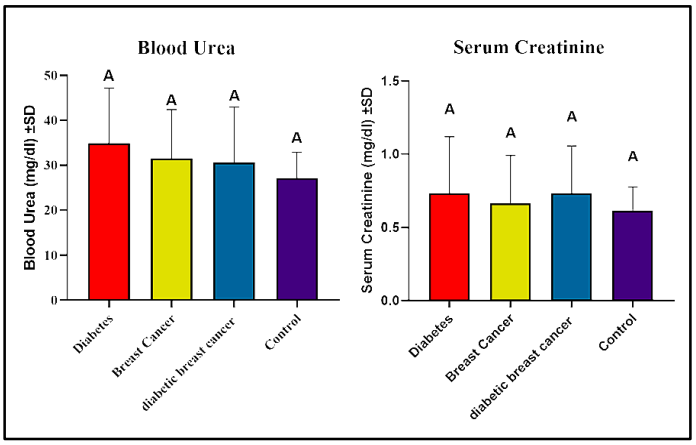

Figure 2: A depiction of the average of control group levels for renal function test (blood urea and serum creatinine) compared with diabetes, breast cancer, and diabetic breast cancer patients.

Liver function test in individuals with diabetes, breast cancer, and diabetic breast cancer compared to control group

Table 4: Comparison of mean values of liver function tests among control group and diabetes, breast cancer, diabetic breast cancer

Characteristic	Diabetes *n* = 30	Breast cancer *n* = 30	Diabetic breast cancer *n* = 30	Control *n* = 30	*p*
ALT (IU/L)					
Mean ±SD	27.8 ± 15.0 A	27.3 ± 10.3 A	24.9 ± 13.7 A	19.7 ± 4.9 B	0.03 O*
Range	10.4 – 61.0	11.7 – 47.0	9.4 – 55.1	13 – 31	
AST (IU/L)					
Mean ±SD	26.8 ± 11.1 A	24.3 ± 8.6 A	26.0 ± 11.5 A	19.0 ± 5.4 B	0.009 O**
Range	12 – 52	12.8 – 45.1	8.1 – 48.6	– 32.1	
ALP (IU/L)					
Mean ±SD	84.3 ± 37 A	87.6 ± 40.1 A	83.4 ± 35.4 A	51.4 ± 15.3 B	0.0003 O***
Range	28 – 234	30 – 188	47 – 201	33 – 84	
TSB (mg/dl)					
Mean ±SD	0.72 ± 0.32 A	0.49 ± 0.19 B	0.48 ± 0.23 B	0.6 ± 0.2 A	0.003 O**
Range	0.3 – 1.7	0.2 – 0.89	0.18 – 1.14	0.3 – 1.1	

n: number of cases; **SD**: standard deviation; **AST**: aspartate aminotransferase; **ALT**: alanine aminotransferase; **ALP**: alkaline phosphatase; **TSB**: total serum bilirubin; O: one-way ANOVA; **NS**: not important; *: noteworthy at $p \leq 0.05$; ***: noteworthy at $p \leq 0.001$. Capital letters **A**, **B** and **C** were used to indicate the level of impact following performance post hoc **LSD** test so that similar letters indicate no significant difference**,** whereas the different letters indicate noteworthy difference.

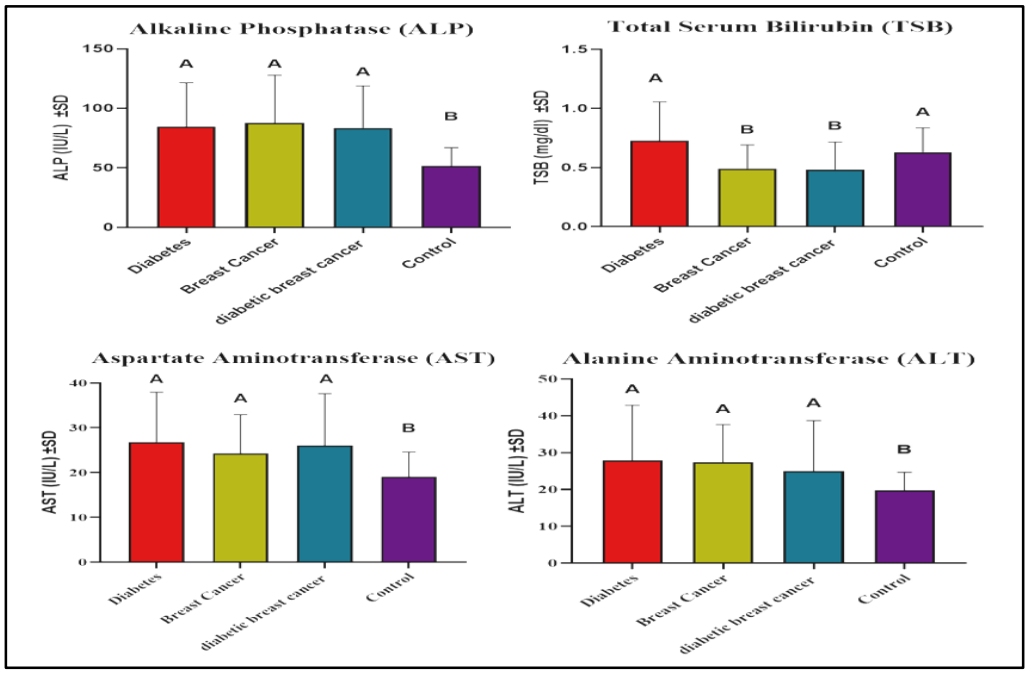

Figure 3: A depiction of the average of control group levels for Liver function test (ALT, AST, ALP, and TSB) compared with diabetes, breast cancer, and diabetic breast cancer patients..

Glucose homeostasis parameters of patients and control group

Table 5: Comparing of Glucose Homeostasis Parameters of different Patients groups and Control Group

Characteristic	Diabetes *n* = 30	Breast cancer *n* = 30	Diabetic breast cancer *n* = 30	Control *n* = 30	
Fasting Blood Sugar (mg/dl)					
Mean ±SD	33.7 ± 63.8 A	93.9 ± 9.9 B	277.6 ± 90.3 C	91.8 ± 9.5 B	0.0001 O***
Range	205 – 460	76 – 125	141 – 433	74 – 117	
Hemoglobin A1c (HbA1c) (%)					
Mean ±SD	9.7 ± 1.3 A	4.8 ± 0.57 B	9.5 ± 1.5 A	5.1 ± 0.5 B	0.0001 O***
Range	7.5 – 12.5	3.8 – 5.8	7.1 – 12.3	4.3 – 6.1	

n: number of cases; **SD**: standard deviation; **O**: one-way ANOVA; **NS**: not noteworthy; *: noteworthy at p ≤ 0.05; ***: noteworthy at p ≤ 0.001. Capital letters **A**, **B** and **C** were used to indicate the level of impact following performance post hoc **LSD** test so that similar letters indicate no significant variance**,** whereas the different letters indicate noteworthy variance.

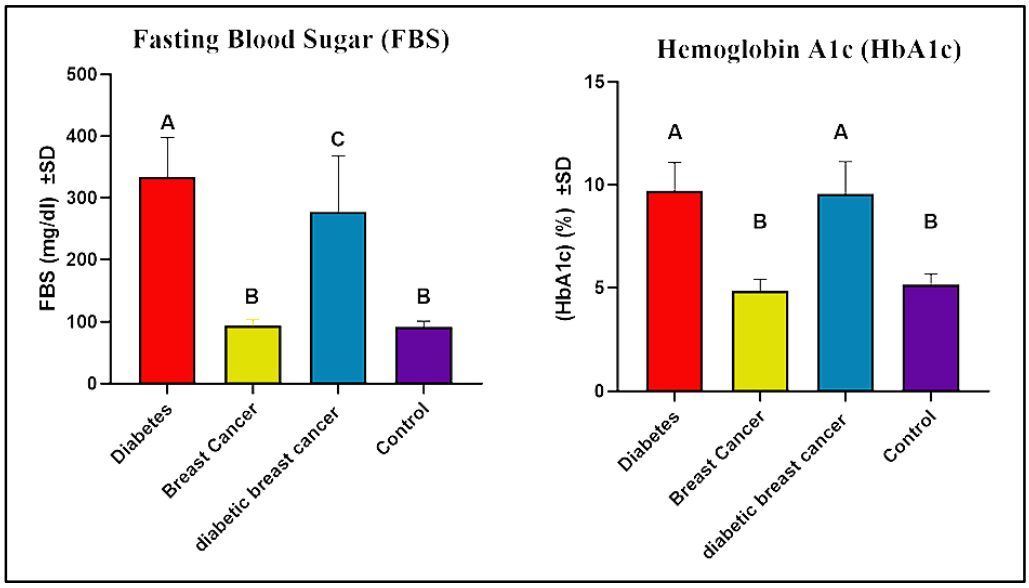

Figure 4: A depiction of the average of control group levels for homeostasis parameters (FBS and HbA1c) compared with diabetes, breast cancer, and diabetic breast cancer patients

Comparison of the mean values of several biomarkers for diabetes, breast cancer, and diabetic breast cancer with those of the control group

Table 6: Comparison of the control group mean values for various biomarkers with those of diabetes, breast cancer, and diabetic breast cancer

Characteristic	Diabetes *n* = 30	Breast cancer *n* = 30	diabetic breast cancer *n* = 30	Control *n* = 30	*P*
GLP-1r (ng/ml)					
Mean ±SD	3.69 ± 1.35 A	3.67 ± 0.55 A	4.2 ± 1.14 A	5.1 ± 2.8 B	0.008 O**
Range	2.74 – 9.54	2.77 – 4.93	2.49 – 7.35	2.75 – 16.5	
AMPK (pg/ml)					
Mean ±SD	23.8 ± 9.1 A	17.9 ± 7.4 A	19.1 ± 10.8 A	31.7 ± 10.1 B	0.0003 O***
Range	3.4 – 46.4	5.65 – 31.1	3.39 – 36.9	4.4 – 55.7	
Caspase -3 (ng/ml)					
Mean ±SD	2.13 ± 0.8 A	2.17 ± 0.47 A	2.54 ± 0.61 A	3.88 ± 1.7 B	0.005 O**
Range	1.14 – 5.59	1.17 – 2.97	1.4 – 3.69	– 8.64	

n: number of cases; **SD**: standard deviation; **GLP-1r**: Glucagon-like peptide-1-receptor; **AMPK**: Adenosine monophosphate activated protein kinase; O: one-way ANOVA; **NS**: not noteworthy; *: noteworthy at $p \leq 0.05$; ***: significant at $p \leq 0.001$. Capital letters **A**, **B** and **C** were used to indicate the level of significance following performance post hoc **LSD** test so that similar letters indicate no significant alteration**,** whereas the different letters indicate significant variance.

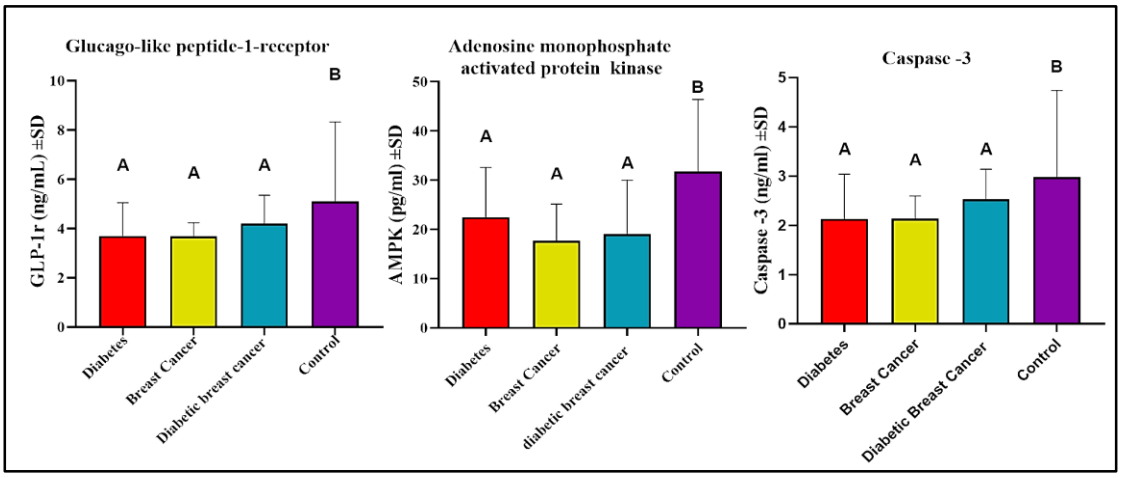

Figure 5: A depiction of the average of control group levels for (Glucagon-like peptide-1-receptor, Adenosine monophosphate activated protein kinase and Caspase-3) biomarkers compared with diabetes, breast cancer, and diabetic breast cancer patients.

Correlations among clinical and biochemical characteristics

Table 7: Correlations among clinical and biochemical characteristics in diabetes

Biomarkers		GLP-1r	AMPK	Caspase -3
ALT (IU/L)	***r* value**	- 0.011	-0.076	-0.180
	***P* value**	0.9	0.7	0.09
AST (IU/L)	***r* value**	0.076	0.133	-0.149
	***P* value**	0.8	0.5	0.1
ALP (IU/L)	***r* value**	0.04	-0.165	0.150
	***P* value**	0.6	0.2	0.1
TSB (mg/dl)	***r* value**	0.09	-0.108	0.166
	***P* value**	0.8	0.4	0.1
B.urea (mg/dl)	***r* value**	0.04	-0.235	-0.06
	***P* value**	0.8	0.05*	0.7
Creatinine (mg/dl)	***r* value**	-0.218	-0.378	-0.219
	***P* value**	0.05*	0.03	0.05
HbA1c (%)	***r* value**	0.107	0.05	0.158
	***P* value**	0.11	0.7	0.1
RBS (mg/dl)	***r* value**	-0.186	-0.09	0.07
	***P* value**	0.09	0.6	0.7
GLP-1r (ng/ml)	***r* value**	1	0.451	0.850
	***P* value**		0.01*	0.001**
AMPK (pg/ml)	***r* value**	0.451	1	0.360
	***P* value**	0.01*		0.03*
Caspase -3 (ng/ml)	***r* value**	0.850	0.360	1
	***P* value**	0.001**	0.03*	

The Pearson test was employed to investigate the relationship. r = correlation coefficient. *Significant correlation at <0.05; **Significant correlation at 0.01. **FBS**: Fasting Blood Sugar; **GLP-1r**: Glucagon-like peptide-1-receptor; **AMPK**: Adenosine monophosphate activated protein kinase; **AST**: aspartate aminotransferase; **ALT**: alanine aminotransferase; **ALP**: alkaline phosphatase; **TSB**: total serum bilirubin.

Table 8: Correlations among clinical and biochemical characteristics in breast cancer

Biomarkers		GLP-1r	AMPK	Caspase -3
ALT (IU/L)	*r* value	- 0.016	0.014	0.163
	P value	0.9	0.9	0.09
AST (IU/L)	*r* value	-0.367	-0.365	0.033
	P value	0.03	0.03	0.9
ALP (IU/L)	*r* value	-0.193	-0.179	-0.103
	P value	0.08	0.09	0.3
TSB (mg/dl)	*r* value	0.112	0.317	0.445
	P value	0.3	0.03	0.01*
B. Urea (mg/dl)	*r* value	-0.365	-0.105	-0.113
	P value	0.03	0.3	0.3
Creatinine (mg/dl)	*r* value	-0.225	0.09	-0.133
	P value	0.05*	0.8	0.1
HbA1c (%)	*r* value	0.326	0.005	-0.019
	P value	0.03	0.9	0.9
RBS (mg/dl)	*r* value	-0.02	-0.235	-0.153
	P value	0.9	0.05*	0.1
GLP-1r (ng/ml)	*r* value	1	0.429	0.306
	P value		0.01*	0.03*
AMPK (pg/ml)	*r* value	0.429	1	0.350
	P value	0.01*		0.03*
Caspase -3 (ng/ml)	*r* value	0.306	0.350	1
	P value	0.03*	0.03*	

The Pearson test was employed to investigate the relationship. r = correlation coefficient. *Significant correlation at <0.05; **Significant correlation at 0.01. **FBS**: Fasting Blood Sugar; **GLP-1r**: Glucagon-like peptide-1-receptor; **AMPK**: Adenosine monophosphate activated protein kinase; **AST**: aspartate aminotransferase; **ALT**: alanine aminotransferase; **ALP**: alkaline phosphatase; **TSB**: total serum bilirubin.

Table 9: Correlations among clinical and biochemical characteristics in diabetic breast cancer

Biomarkers		GLP-1r	AMPK	Caspase -3
ALT (IU/L)	*r* value	- 0.238	-0.393	-0.612
	P value	0.05*	0.03*	0.004**
AST (IU/L)	*r* value	-0.09	0.08	0.109
	P value	0.7	0.9	0.1
ALP (IU/L)	*r* value	-0.288	0.215	-0.218
	P value	0.05*	0.05*	0.05*
TSB (mg/dl)	*r* value	0.303	-0.225	0.085

	P value	0.04*	0.05*	0.9
B. Urea (mg/dl)	*r* value	-0.241	0.175	0.138
	P value	0.05*	0.09	0.1
Creatinine (mg/dl)	*r* value	0.220	0.003	0.160
	P value	0.05*	0.9	0.1
HbA1c (%)	*r* value	0.08	0.313	0.165
	P value	0.7	0.04	0.1
RBS (mg/dl)	*r* value	0.185	0.125	-0.008
	P value	0.09	0.1	0.9
GLP-1r (ng/ml)	*r* value	1	0.323	0.513
	P value		0.03*	0.009**
AMPK (pg/ml)	*r* value	0.323	1	0.538
	P value	0.03*		0.009**
Caspase -3 (ng/ml)	*r* value	0.513	0.538	1
	P value	0.009**	0.009**	

The Pearson test was employed to investigate the relationship. r = correlation coefficient. *Significant correlation at <0.05; **Significant correlation at 0.01. **FBS**: Fasting Blood Sugar; **GLP-1r**: Glucago-like peptide-1-receptor; **AMPK**: Adenosine monophosphate activated peotein kinase; Glucagon-like peptide -1; **AST**: aspartate aminotransferase; **ALT**: alanine aminotransferase; **ALP**: alkaline phosphatase; **TSB**: total serum bilirubin.

Table 10: Receiver-operating characteristic (ROC) curve analysis of biomarkers for diabetic patient group.

Characteristic	Cut off value	AUC	95 % CI	*p*	Sensitivity %	Specificity %	Accuracy %
GLP-1r (ng/ml)	> 3.75	0.82	0.689 to 0.951	0.0003	81.8	77.3	82.1
AMPK (pg/ml)	> 29.87	0.68	0.524 to 0.851	0.03	60	81.8	68.8
Caspase -3 (ng/m	> 2.46	0.64	0.476 to 0.821	0.09	59	72.7	64.9

GLP-1r: Glucago-like peptide-1-receptor; **AMPK**: Adenosine monophosphate activated protein kinase; **AUC**: area under curve; **CI**: confidence interval; ***: significant at p ≤ 0.001

Table 11: Receiver-operating characteristic (ROC) curve analysis of biomarkers for breast cancer patient group.

Characteristic	Cut off value	AUC	95 % CI	*p*	Sensitivity %	Specificity %	Accuracy %
GLP-1r (ng/ml)	> 3.8	0.75	0.607 to 0.90	0.0039	68.18	72.73	75.4
AMPK (pg/ml)	> 29.8	0.78	0.637 to 0.924	0.0014	60	95.45	78.5
Caspase -3 (ng/ml)	> 2.63	0.58	0.415 to 0.762	0.31	45.5	72.7	58.8

GLP-1r: Glucago-like peptide-1-receptor; **AMPK**: Adenosine monophosphate activated protein kinase; **AUC**: area under curve; CI: confidence interval; ***: significant at p ≤ 0.001

Table 12: Receiver-operating characteristic (ROC) curve analysis of biomarkers for diabetic breast cancer group.

Characteristic	Cut off value	AUC	95 % CI	*p*	Sensitivity %	Specificity %	Accuracy %
GLP-1r (ng/ml)	> 4.6	0.58	0.413 to 0.752	0.33	36.3	72.7	58.4
AMPK (pg/ml)	> 30.7	0.75	0.605 to 0.894	0.0045	50	72.7	75
Caspase -3 (ng/ml)	< 2.732	0.57	0.403 to 0.749	0.38	59.1	54	57.6

GLP-1r: Glucagon-like peptide-1-receptor; **AMPK**: Adenosine monophosphate activated protein kinase; **AUC**: area under curve; CI: confidence interval; ***: significant at p ≤ 0.001

DISCUSSION

The average age of the diabetic patients in this study is similar to the results of (Al-Musawi, 2021), who discovered that the average age was 52 years (Al-Musawi et al., 2021) High body mass index (BMI) is a significant risk factor for the development of type 2 diabetes, as demonstrated by the current study, where the BMI of the patients' group was significantly greater than that of the control group (Y. Wang et al., 2022; Kanval et al., 2024). Obesity, especially central obesity (extra fat around the abdomen), is intimately associated with diabetes mellitus. According to Akinbami et al., WBC counts were greater in breast cancer patients than in controls, which is consistent with the findings of the current investigation (Akinbami et al., 2013). In a large case-control study, the WBC counts of 4,402 breast cancer patients and 4,402 propensity score-matched controls, who were chosen from the Korean National Health and Nutrition Examination Survey, were compared. The results clearly contradicted the current study's findings, as the mean WBC count of the patient group was significantly lower than that of the control group(Lu et al., 2006). A high WBC count has been associated with the incidence and mortality of cancer, as well as atherosclerotic cardiovascular diseases, even when it is within the normal range (Anderson et al., 2012; Rashid et al., 2023). Additionally consistent with the findings of the current investigation, Adane et al. found that individuals with diabetes mellitus had higher WBC counts than controls (Adane et al., 2021). A large body of research indicates that renal impairment is common in cancer patients. According to reports, renal insufficiency is associated with a lower overall survival rate and a greater death rate from cancer. Accordingly, it is essential to use a reliable and precise method of assessing renal function to check for renal inefficiency in cancer individuals (Wanchoo et al., 2016). Patients with diabetes who have impaired levels of urea and creatinine as a result of elevated blood glucose have reduced renal function (Kamal, 2014; Jam et al., 2013). Hasbahceci's study involved measuring the levels of blood urea and serum creatinine in a subject of breast cancer patients and comparing them to a control subject. The findings showed that there was no statistically significant difference in the mean levels of these two parameters between the two groups (Hasbahceci et al., 2018; Jam et al., 2014). As a result, the current study and Hasbahceci's agree. The current investigation found that the sick group's ALT, AST, ALP, and TSB were considerably higher than those of the control group. It was determined that diabetes and cancer were serious, long-term illnesses. Diabetes patients have been linked to an increased incidence of liver cancer, among other cancers, according to epidemiological research. Because of complicating factors such the length of diabetes, the medications used for treatment, and complications from the condition, it is challenging to precisely determine the cancer risk in people with diabetes (Vigneri et al., 2020). There have also been reports of elevated alkaline phosphatase levels, which are caused by either direct drug damage to the bile ductular cells or indirect damage caused by an adaptive immune response (Dara et al., 2020). At the time of presentation, 92% of patients had poor liver function tests; the most frequently increased enzymes were alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT). Furthermore, aspartate transaminase

(AST) levels greater than twice the upper limit of normal were present in 54% of all patients (Schatka et al., 2021).

In the study that was presented, the patient group's FBS and HbA1c were considerably greater than those of the control group. High HbA1c values are a sign of inadequate long-term glucose management. Cardiovascular disorders are responsible for between 50 and 75 percent of diabetes-related deaths (Lachin et al., 2021). Elevated HbA_1c has been regarded as an independent risk factor for CAD in subjects with or without diabetes (Khan et al., 2023). A 1% decrease in HbA1c was linked to a 21% lower risk of any diabetes-related end point, a 21% lower risk of diabetes-related mortality, a 14% lower risk of myocardial infarction, and a 37% lower risk of microvascular complications (Almzaiel et al., n.d.; Kadooh et al., 2024). Increased blood viscosity has been linked to an increase in HbA1c, which is seen in situations where diabetic management is inadequate. Increased blood viscosity is caused by hemoglobin's glycosylation and elevated glucose levels, which tend to alter red blood cell characteristics by decreasing the cells' flexibility and enhancing their propensity to aggregate(Sherwani et al., 2016). In the study that was presented, the patient group's levels of GLP-1r, AMPK, and Caspase-3 were considerably lower than those of the control group. Insulin resistance may be the cause of noticeably reduced GLP-1 receptor (GLP-1r) expression or activity in diabetic patients, especially those with type 2 diabetes (Holst et al., 2021). Insulin resistance-related hyperinsulinemia can cause the downregulation of GLP-1 receptors, which lowers their expression (Bednarz et al., 2022; Haddawi et al., n.d.). It is unknown what is causing the relative decline in GLP-1R expression in T2DM patients. The possibility that it stems from hyperglycemia itself is suggested by the finding that short-term hyperglycemia causes lower GLP-1R expression in the β-cells of hyperglycemic rats (Kaneto et al., 2021) GLP-1 signaling pathways can be disrupted by chronic inflammation, which is frequently seen in obesity and type 2 diabetes. A vital regulator of cellular energy homeostasis, AMP-activated protein kinase (AMPK) is involved in the metabolism of fats and carbohydrates. AMPK regulates whole-body glucose homeostasis (Mayssam Makki Salih et al., 2023; Schimmack et al., 2006). Free fatty acids have the ability to cause intracellular lipid buildup and stress kinase activation, both of which reduce AMPK action. A major contributing factor to the development of obesity and insulin resistance is dietary excess(Li et al., 2014; Mayssam M. Salih et al., 2024). An enzyme called caspase-3 is involved in apoptosis, or programmed cell death. According to recent research, diabetes can alter gene expression and cellular behaviour only through hyperglycemia's generation of reactive oxygen species (ROS) in the mitochondria. Because elevated oxidative stress disrupts apoptotic signaling pathways, it can prevent caspase-3 activation and apoptosis (Abed et al., 2023; Sangaran et al., 2021).

CONCLUSION

Diabetes patients had lower levels of caspase-3, AMPK, and GLP-1 receptor, which highlights the complex interplay between cellular death, compromised signaling pathways, and metabolic dysfunction. Knowing these molecular changes could help us better understand the pathophysiology of diabetes and open up new treatment options that target reestablishing these pathways to slow down the progression of the illness. Furthermore, the reduced expression of caspase-3, AMPK, and GLP-1 receptor in breast cancer patients raises the possibility that these signaling molecules play a role in the initiation and spread of tumours. This demonstrates the intricate relationships that exist between cancer biology, cellular metabolism, and metabolic dysregulation.

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Ethical considerations:

All parents or caregivers of the participating patients provided their signature on a consent form to take samples. The study adhered to the ethical guidelines outlined in the declaration of Helsinki (1964) for medical research involving human participants. Ethical approval for the study was obtained from the Ethical and Research Committee of the Department of Medical Chemistry, College of Medicine, University of Al-Qadisiyah, and Al Sadr Teaching Hospital Iraq.

Conflicts of interest:

The authors declare that they have no competing interests and no conflicts of interest

Consent of Patient

All participants provided written informed consent prior to enrollment in the study. The study protocol was reviewed and approved by the Institutional Review Board of the University of Al-Qadisiyah, Iraq. Participants were informed about the purpose of the study, the procedures involved, and the potential risks and benefits. They were assured that their personal information would be kept confidential and that they had the right to withdraw from the study at any time without penalty. Blood samples were obtained by trained phlebotomists using standard aseptic techniques. Participants did not receive any compensation for their participation. The study was conducted in accordance with the principles of the Declaration of Helsinki.

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