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Evaluating Serum Biomarkers (DPP-4, GLP-1, Ki-67 Protein) Levels for Early Detection of Diabetic Breast Cancer

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ABSTRACT

Background: *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 perseverance ratio for people with mammary neoplasms 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 characterized by elevated blood sugar levels brought on by flaws in the body's capacity to make and/or utilize insulin.* ***Objectives:*** *This study aimed to evaluate the levels of serum biomarkers (DPP-4, GLP-1, Ki-67 protein) in individuals with diabetes, breast cancer, diabetic breast cancer, and a control group.* ***Methods****: The samples were taken from individuals at Al-Najaf Teaching Hospital and the Najaf Cancer Centre between 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 consisted of 30 participants, (Diabetes type 2 patient group, Breast cancer patient group, Diabetic breast cancer patient group, and Control group).* ***Results:*** *The difference in serum DPP-4 levels, between diabetic, breast cancer individuals and Healthy control groups, is statistically noteworthy. The results show a higher DPP-4 level in patient groups than in the control group. While no significance in the GLP-1 and Ki-67 proteins in the patient and control groups.* ***Conclusion:*** *These results suggest that high serum DPP-4 could serve as a useful screening marker for the early detection of diabetic breast cancer. While, The lack of differences in GLP-1 and Ki-67 proteins levels across the groups indicates these biomarkers may not be as informative for identifying diabetic breast cancer in this population.*

Keywords: Diabetic Breast Cancer; Serum Biomarkers; DPP-4; GLP-1; Ki-67 protein; Early Detection.

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INTRODUCTION

Mammary cancer is one of the most predominant human malignancies, There are many diverse tumour subtypes, risk factors, and treatment options for mammary malignancy (1). Mammary cancer develops when malignant cells proliferate. When cancerous cells form in the lining of the mammary milk-producing glands or ducts (ductal epithelium), the condition is categorized as cancer. What sets cancer cells apart from other types of cells is their capacity to attack surrounding healthy tissue or move all through the body, a process identified as metastasis. They can also proliferate

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uncontrollably, resulting in aberrant development (2). Mammary cancer is the most common cancer in the world that murders and influences women (3). Males can also cultivate mammary cancer, while ladies are more than 100 times more expected to do so than guys. In America, there will be 42,260 mammary malignancy decreases in 2019, according to estimates(4). Currently, after cardiovascular diseases, breast malignancy is the second largest cause of death for females in Iraq(5).

However, according to the Iraqi Cancer Council, the percentage of women with breast malignancy is 34.06% greater than that of other cancer types; that is, the disease claimed the lives of 23.02% of Iraqi women in 2018 and had an infection rate of 32.31 per 100,000 or 6,018 per 100,000. Additionally, the prevalence of the disease is increasing among women between the ages of 35 and 75 (6). Age, menstruation, alcohol use, hormonal factors, inadequate nutrition, environmental factors, hereditary factors, obesity, and other factors all increase the risk of developing breast malignancy. The exact causes of breast malignancy are not well understood, and they are not caused by a single cause (7). Males only have rudimentary mammary glands, yet both sexes have mammary glands. In females, they reach full development following puberty. Each breast projects a gently rounded protrusion from the pectoral area. The skin above the middle of the elevation displays a circular area called the areola that is deeply pigmented. The nipple is a protrusion that protrudes from the middle of the areola(8). 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.

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 (9). This illness is predominantly recognized by the degree of hyperglycemia that raises the risk of microvascular injury (retinopathy, nephropathy, and neuropathy) (10). It is related to a reduced life expectancy, significant injury from certain diabetes-related microvascular illnesses, an increased risk of macrovascular consequences (peripheral vascular disease, ischemic heart disease, and stroke), and a worse quality of life (11).

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(12). 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 (13). Diabetes mellitus is commonly characterized by thirst, polyuria, blurred vision, and weight loss. Many times, the symptoms are negligible or nonexistent (14).

In 2006, DPP-4 inhibitors were introduced as a type 2 diabetes medication. Through an increase in endogenous GLP-1 concentrations, they suppress glucagon secretion and boost insulin secretion without posing an inherent risk of hypoglycemia (15). DPP-4 inhibitors are tiny, active compounds that can be taken orally that inhibit DPP-4. The endogenous GLP-1 concentration is increased two to three times when DPP-4 inhibitors are administered. High-affinity substrate GLP-1 has for DPP-4, often known as the "direct target." In addition to GLP-1, DPP-4 has additional substrates, and its increase through DPP-4 suppression may also be an "indirect target" or "off-target" factor in the normalization of glycemic levels in type 2 diabetes (16). The endogenous elevation of GLP-1's incretin hormone concentration, which

subsequently causes a glucose-dependent initiation of insulin secretion and a suppression of glucagon excretion, is the therapeutically most significant and relevant activity of DPP-4 inhibitors (17). All DPP-4 suppression can be given in a typical dose without the need for dose titrations. GLP-1 has the highest substrate specificity for DPP-4, but DPP-4 has additional substrates in addition to inhibition therefore spreading the biological half-life of the incretin hormone GIP, brain natriuretic peptide, and many others (18). GLP-1, also known as glucagon-like peptide-1, is a complex hormone with a wide range of therapeutic applications. GLP-1 has a wide range of metabolic effects, including modulating the proliferation of rodent β-cells, increasing natriuretic and diuresis, decreasing stomach emptying, inhibiting food intake, and stimulating insulin secretion in a glucose-dependent manner (19). The monoclonal antibody Ki-67, which was produced by immunizing mice with the nucleus of the Hodgkin's lymphoma cell line L248—which was initially discovered by Gerdes et al. All vertebrates express the nuclear DNA binding protein Ki-67, which is a commonly used proliferation marker for cancer grading (20).

MATERIALS AND METHODS

Subjects

The study included 120 women participants, who were divided into four groups which are the experimental group consisted of 30 participants, Diabetes type 2 patient group, Breast cancer patient group, Diabetic breast cancer patient group, and Control group**.** 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 were 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 among control subjects and individuals with diabetes, breast cancer, and diabetic breast cancer.

Characteristics	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	0.06 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	0.0256 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 noteworthy;* : noteworthy at $p \leq 0.05$. 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 differences.

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.

Characteristics	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; *: noteworthy at $p \leq 0.05$; ***: noteworthy at $p \leq 0.001$. Principal cultivations **A**, **B** and **C** were used to show the level of impact following the performance post hoc **LSD** test so that comparable letters indicate no important variance**,** while the diverse letters indicate noteworthy alteration **(Figure 1).**

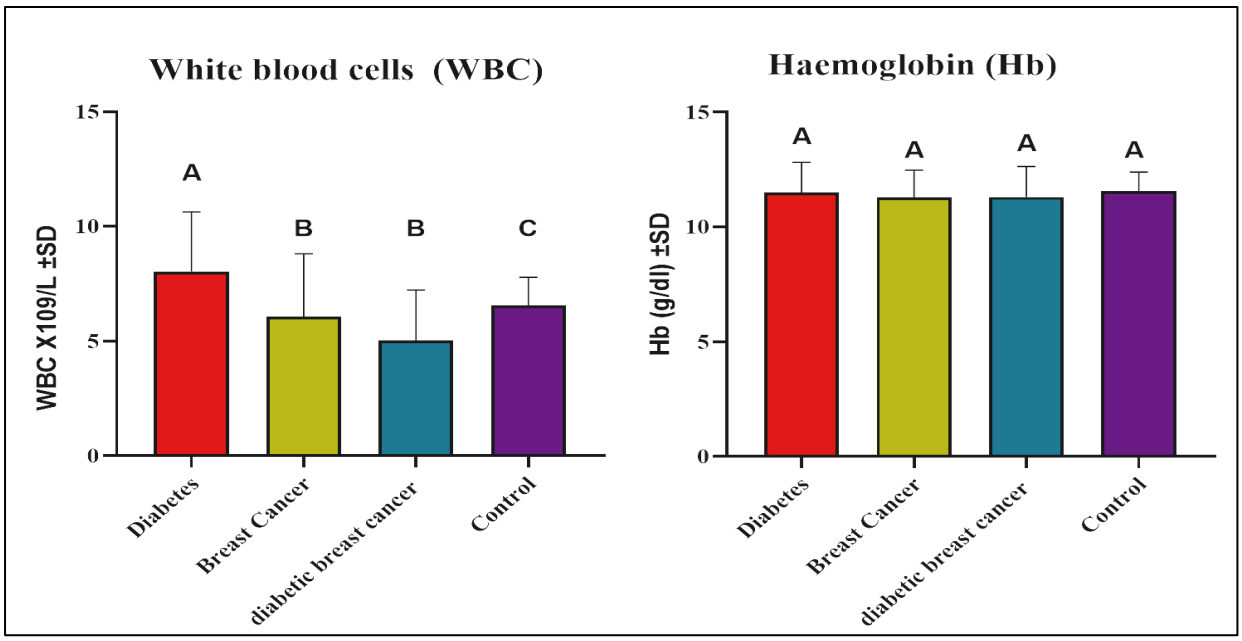

Figure 1: A depiction of the average of control group levels for hematological characteristics (White blood cell (WBC) and Haemoglobin (Hb)) compared with diabetes, breast cancer, and diabetic breast cancer patients.

Comparison of blood Urea and serum Creatinine among control subjects and individuals with diabetes, breast malignancy, and diabetic breast cancer:

Table 3: Comparison of blood urea and serum creatinine among individuals with diabetes, mammary malignancy, diabetic breast malignancy, and control subject

Characteristics	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 noteworthy; *: noteworthy at $p \leq 0.05$; ***: noteworthy at $p \leq 0.001$. Wealth letters **A**, **B** and **C** were used to show the level of impact following performance post hoc **LSD** test so that comparable letters show no significant alteration**,** whereas the dissimilar letters indicate noteworthy variance (**Figure 2)**.

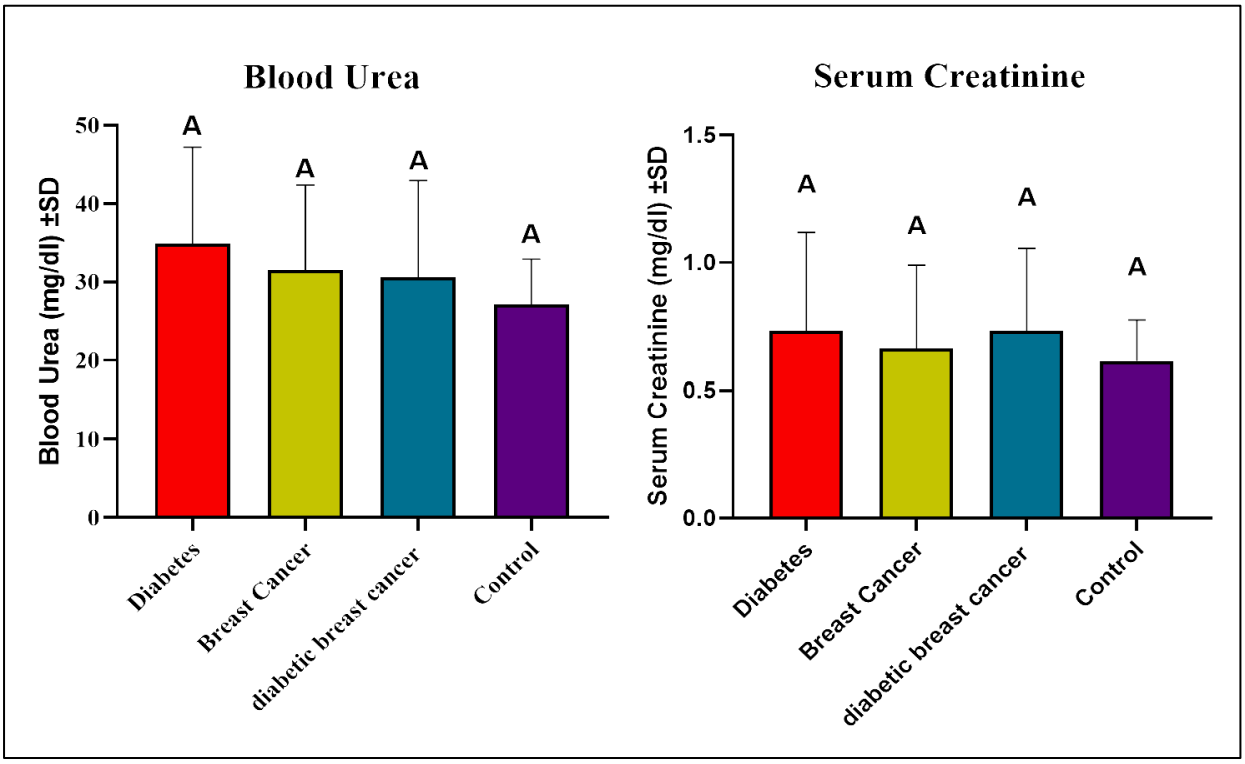

Figure 2: A depiction of the average of control group levels for renal function test (blood urea and serum creatinine) compared with diabetes, breast cancer, and diabetic breast cancer patients.

Liver function test in individuals with Diabetes, Breast carcinoma, and Diabetic Breast carcinoma compared to a healthy group:

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

Characteristics	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
Range	12 – 52	12.8 – 45.1	8.1 – 48.6	12.8 – 32.1	**O****
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
Range	28 – 234	30 – 188	47 – 201	33 – 84	**O*****
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
Range	0.3 – 1.7	0.2 – 0.89	0.18 – 1.14	0.3 – 1.1	**O****

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 alteration**,** whereas the diverse letters indicate noteworthy difference **(Figure 3).**

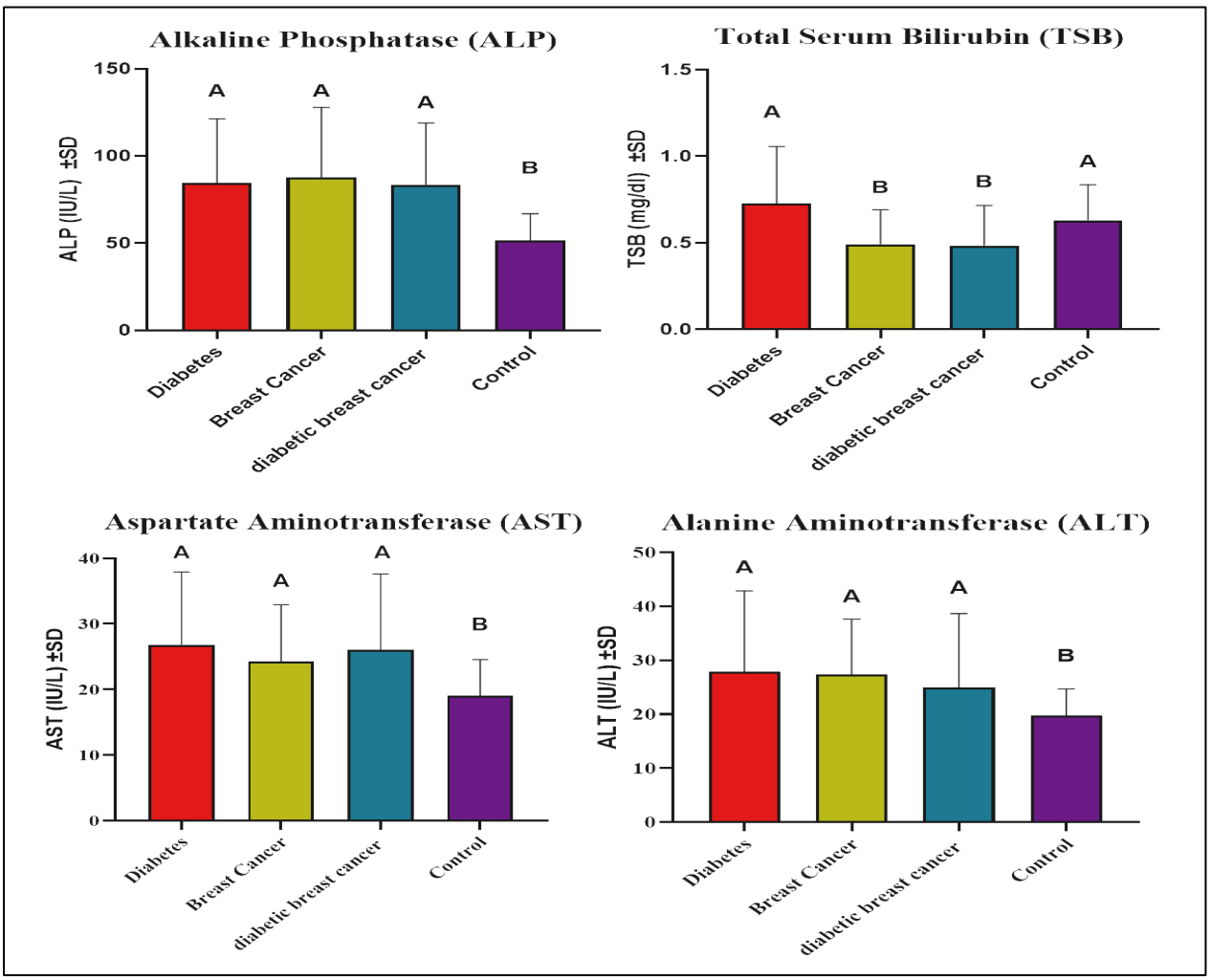

Figure 3: A depiction of the average of control group levels for Liver function test (ALT, AST, ALP, and TSB) compared with diabetes, breast cancer, and diabetic breast cancer patients.

Glucose homeostasis parameters of patients and control group:

Table 5: Comparing of Glucose homeostasis parameters of mean values of liver function tests among control group and diabetes, breast cancer, diabetic breast cancer:

Characteristics	Diabetes n = 30	Breast cancer n = 30	Diabetic Breast cancer n = 30	Control n = 30	p
Fasting Blood Sugar (mg/dl)					
Mean ±SD	333.7 ± 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 $p \leq 0.05$; ***: noteworthy at $p \leq 0.001$. Principal letters **A**, **B** and **C** were utilized to show the level of impact

following performance post hoc **LSD** test so that comparable letters indicate no important variance**,** while the diverse letters indicate noteworthy variance (**Figure 4**).

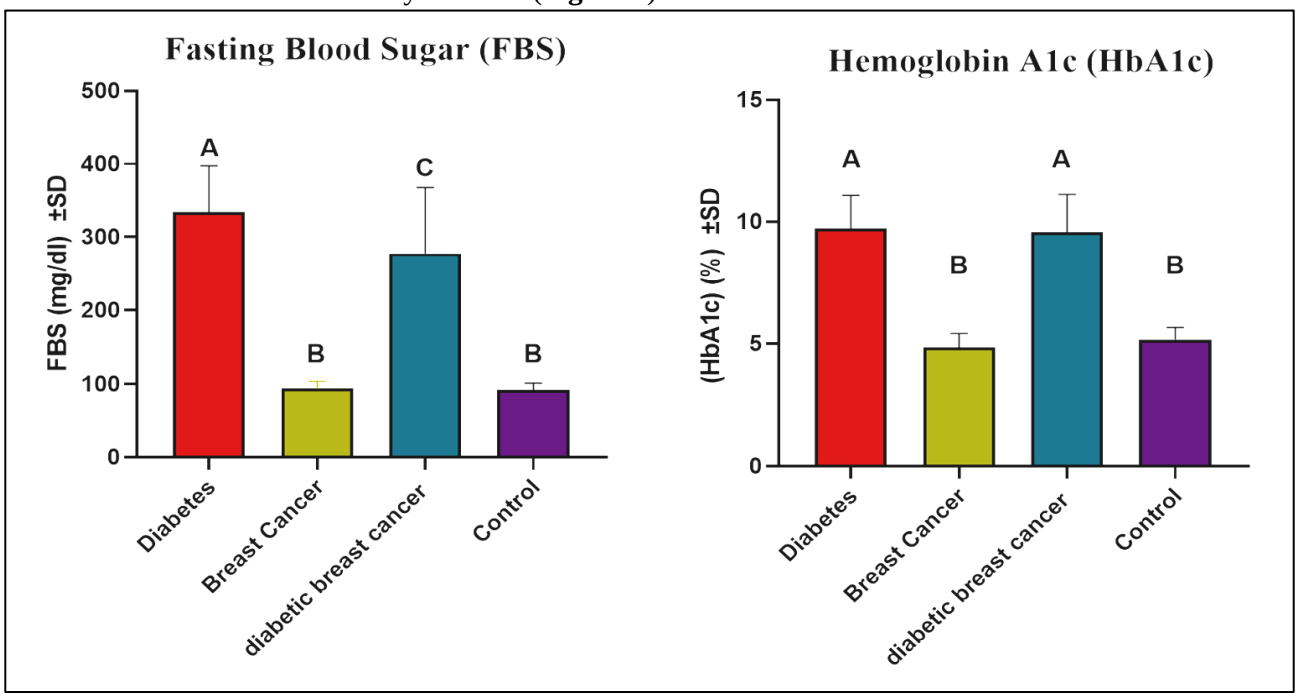

Figure 4: A depiction of the average of control group levels for homeostasis parameters (FBS and HbA1c) compared with diabetes, breast cancer, and diabetic breast cancer patients.

Comparison of the mean values of several biomarkers for diabetes, breast carcinoma, and diabetic breast carcinoma 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.

Characteristics	**Diabetes n = 30**	**Breast Cancer n = 30**	**Diabetic Breast cancer n = 30**	**Control n = 30**	**p**
DPP-4 (ng/ml)					
Mean ±SD	136.4 ± 25.3A	136.4 ± 21.3 A	166.9 ± 35 A	141.8 ± 67.6 B	0.0021 O*
Range	101 – 209.7	105 – 177.3	93.4 – 238.5	72.6 – 418	
GLP-1 (pmol/L)					
Mean ±SD	17.2 ± 5.65 A	14.9 ± 2.7 A	17 ± 8.03 A	18.2 ± 7.1 A	0.09 O NS
Range	9.45 – 36.3	9.9 – 19.9	8.1 – 44.4	10.5 – 41.2	
Ki-67 protein (ng/ml)					
Mean ±SD	4.6 ± 1.1 A	4.98 ± 1.3 A	5.39 ± 1.5 A	5.7 ± 2.8 A	0.2 O NS
Range	3.12 – 8.1	3.1 – 7.89	2.8 – 7.86	2.75 – 14.19	

n: number of cases; **SD**: standard deviation; **DPP-4:** Dipeptidyl peptidase-4; **GLP-1**: Glucagon-like peptide - 1; and **Ki-67** protein. O: one-way ANOVA; **NS**: not noteworthy; *: noteworthy at $p \leq 0.05$; ***: noteworthy at $p \leq 0.001$. Principal letters **A**, **B** and **C** were used to show the level of impact following performance post

hoc **LSD** test so that comparable letters show no important alteration**,** while the dissimilar letters signpost important alteration (**Figure 5**).

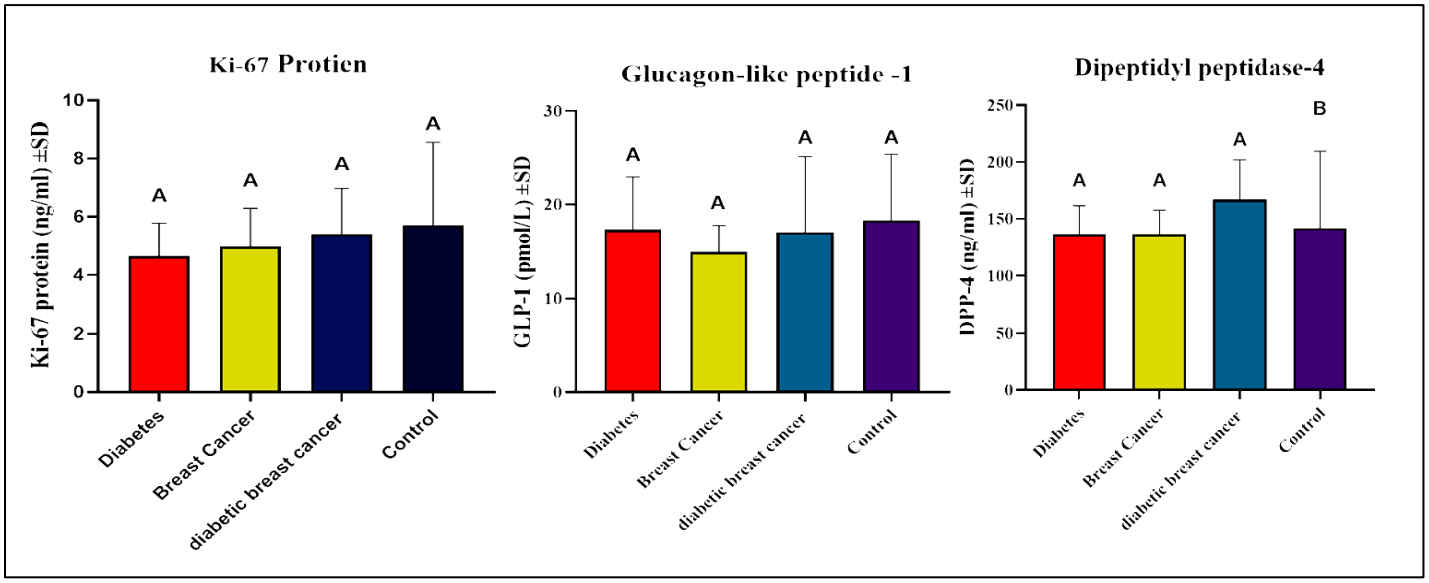

Figure 5: A depiction of the average of control group levels for (DPP-4: Dipeptidyl peptidase-4; GLP-1: Glucagon-like peptide -1; and Ki-67 protein) biomarkers compared with diabetes, breast cancer, and diabetic breast cancer patients.

Correlations between clinical and biochemical characteristics:

Table 7: Correlations among clinical and biochemical characteristics in diabetes

Biomarkers / Diabetes		DPP-4	GLP-1	Ki-67 protein
ALT (IU/L)	r value	0.07	-0.223	-0.111
	P value	0.7	0.05*	0.3
AST (IU/L)	r value	0.09	-0.026	-0.02
	P value	0.6	0.9	0.9
ALP (IU/L)	r value	0.026	0.126	0.141
	P value	0.9	0.1	0.1
TSB (mg/dl)	r value	0.213	0.154	0.104
	P value	0.05*	0.1	0.3
B. Urea (mg/dl)	r value	0.108	0.140	0.176
	P value	0.1	0.1	0.09
Creatinine (mg/dl)	r value	-0.07	0.07	-0.08
	P value	0.7	0.7	0.9
HbA1c (%)	r value	0.09	-0.02	-0.08
	P value	0.6	0.9	0.9
RBS (mg/dl)	r value	-0.140	-0.133	-0.213
	P value	0.1	0.2	0.05*
DPP-4 (ng/ml)	r value	1	0.807	0.896
	P value		0.001**	0.001**
GLP-1 (pmol/L)	r value	0.807	1	0.849
	P value	0.001**		0.001**
Ki-67 protein (ng/ml)	r value	0.896	0.849	1
	P value	0.001**	0.001**	

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; **DPP-4:** Dipeptidyl peptidase-4; **GLP-1**: Glucagon-like peptide -1; **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/ Breast Cancer		DPP-4	GLP-1	Ki-67 protein
ALT (IU/L)	r value	0.078	-0.230	-0.140
	P value	0.7	0.05*	0.2
AST (IU/L)	r value	-0.038	-0.156	-0.431
	P value	0.8	0.1	0.01*
ALP (IU/L)	r value	0.012	-0.326	-0.04
	P value	0.9	0.03*	0.9
TSB (mg/dl)	r value	0.076	0.04	0.06
	P value	0.7	0.9	0.7
B. Urea (mg/dl)	r value	0.10	-0.333	-0.094
	P value	0.3	0.03*	0.9
Creatinine (mg/dl)	r value	-0.116	-0.192	-0.03
	P value	0.3	0.07	0.9
HbA1c (%)	r value	0.04	0.338	0.276
	P value	0.8	0.03*	0.05*
RBS (mg/dl)	r value	0.110	-0.196	0.02
	P value	0.3	0.07	0.9
DPP-4 (ng/ml)	r value	1	0.494	0.08
	P value		0.01*	0.9
GLP-1 (pmol/L)	r value	0.494	1	0.297
	P value	0.01*		0.05*
Ki-67 protein (ng/ml)	r value	0.08	0.297	1
	P value	0.9	0.05*	

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; **DPP-4:** Dipeptidyl peptidase-4; **GLP-1**: Glucagon-like peptide -1; **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/ Diabetic Breast cancer		DPP-4	GLP-1	Ki-67 protein
ALT (IU/L)	r value	-0.510	-0.150	-0.444
	P value	0.009**	0.1	0.01*
AST (IU/L)	r value	0.007	0.246	0.016
	P value	0.8	0.05*	0.9
ALP (IU/L)	r value	-0.193	0.514	-0.289
	P value	0.07	0.009**	0.05*
TSB (mg/dl)	r value	-0.013	0.343	-0.03
	P value	0.8	0.03*	0.8
B. Urea (mg/dl)	r value	0.169	0.409	0.286
	P value	0.1	0.01*	0.05*
Creatinine (mg/dl)	r value	0.208	-0.02	0.217
	P value	0.05*	0.9	0.05*
HbA1c (%)	r value	0.154	0.420	0.222
	P value	0.1	0.01*	0.05*
RBS (mg/dl)	r value	0.110	0.213	0.307
	P value	0.3	0.05*	0.04*
	r value	1	0.389	0.796

DPP-4 (ng/ml)	**P value**		**0.01***	**0.004****
GLP-1 (pmol/L)	**r value**	**0.389**	**1**	**0.211**
	P value	**0.01***		**0.05***
Ki-67 protein (ng/ml)	**r value**	**0.796**	**0.211**	**1**
	P value	**0.004****	**0.05***	

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; **DPP-4:** Dipeptidyl peptidase-4; **GLP-1**: 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.

Characteristics	Cut off value	AUC	95 % CI	p	Sensitivity %	Specificity %	Accuracy %
DPP-4 (ng/ml)	< 124.7	0.52	0.353 to 0.70	0.7	37	68	52.7
GLP-1 (pmol/L)	< 13.39	0.59	0.42 to 0.768	0.2	40.9	81.2	59.7
Ki-67 protein (ng/ml)	> 5.7	0.58	0.409 to 0.752	0.3	31	86.4	58.1

DPP-4: Dipeptidyl peptidase-4; **GLP-1**: Glucagon-like peptide -1; **AUC**: Area under curve; **CI**: Confidence interval; ***: significant at $p \leq 0.001$.

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 %
DPP-4 (ng/ml)	< 136.0	0.58	0.414 to 0.758	0.32	59.6	50	58.6
GLP-1 (pmol/L)	> 15.39	0.57	0.399 to 0.745	0.41	45.5	72.7	57.2
Ki-67protein (ng/ml)	> 7.210	0.51	0.339 to 0.689	0.8	18	95.4	51.4

DPP-4: Dipeptidyl peptidase-4; **GLP-1**: Glucagon-like peptide -1; **AUC**: area under curve; CI: confidence interval; ***: significant at $p \leq 0.001$.

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 %
DPP-4 (ng/ml)	< 133.0	0.67	0.5158 to 0.8437	0.04	59.1	77.1	67.9
GLP-1 (pmol/L)	< 15.55	0.5	0.334 to 0.682	0.9	54.5	50	50.8
Ki-67 protein (ng/ml)	< 4.728	0.54	0.371 to 0.719	0.6	50	59.1	54.5

DPP-4: Dipeptidyl peptidase-4; **GLP-1**: Glucagon-like peptide -1; **AUC**: area under curve; CI: confidence interval; ***: significant at $p \leq 0.001$.

DISCUSSION

The average age of the diabetic patients in this study is similar to the results of Al-Musawi, Al-Lami, and Al-Saadi 2021, who discovered that the average age was 52 years (21), and Marzoq, Mohammed, and Habib 2020 who collected registered data from Al Fayha Diabetic Foot Clinic, Basrah, Iraq. from January 2018 through July 2019. There were 73 adult patients with diabetic foot ulcers in it; there were 29 male patients (39.7%) and 44 female patients (60.3%). Moreover, they discovered that the average age was 52 (22). 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 (23).

The disease known as hepatic steatosis, in which fat builds up in the liver cells, can be brought on by this extra fat. Insulin resistance, a disorder where the body's cells don't react to the hormone insulin (which controls blood sugar levels) as well, is linked to obesity (24). 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 (25). However, according to Park et al (26), 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 contradicted the current study's findings, as the mean WBC count of the patient group was significantly lower than that of the control group. There is growing evidence that the etiology of some cancers may be influenced by chronic low-grade inflammation (27). Additionally consistent with the findings of the current investigation, Adane et al. found that individuals with diabetes mellitus had higher WBC counts than controls (28). Diabetes can affect various systems in the body, including the immune system (29), Since obesity is frequently linked to both inflammation and insulin resistance, chronic inflammation is common in diabetes (30), Through varying glucose and lipid metabolism in skeletal muscle, liver, and adipose tissue, they significantly worsen human health (31)(32).

There was no discernible difference in haemoglobin levels between the patient and control groups, according to this study. Systemic inflammation, inhibition of erythropoietin release, renal interstitium injury, severe symptomatic autonomic neuropathy initiating efferent sympathetic denervation of the kidney and loss of suitable erythropoietin, medications, altered iron metabolism, and hyperglycemia are some of the factors that have been proposed as the cause of the former onset of anaemia in diabetic patients (33). Moreover, anaemia in malignancy individuals is strongly linked with both the illness itself (34)(35) and the kind of malignancy (36).

Additionally, the effects of malignancy (by direct attack of the bone marrow), cancer management (surgery, hormone therapy, radiation, and targeted therapy), and the influence of cytokines generated by malignant cells all led to a rise in the incidence of anaemia in cancer patients (37). 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 (38). 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 (39). As a result, the current study and Hasbahceci's agree. Furthermore, El-attar's findings, which

showed no appreciable variation in blood urea and serum creatinine levels concerning women with mammary cancer and the control subject, are in line with the findings of the current study (40).

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 (41). These enzymes seep into the circulation when liver cells are injured or irritated, raising blood levels (42). Higher levels of these enzymes often correlate with more severe forms of NAFLD (43).

Individuals receiving chemotherapy have moderately good overall predictions, with many persisting for a median of 13 months (44). Hepatic metastasis has been found in 55% to 75% of all autopsies performed on patients who died from breast malignancy (45). 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 (46). For breast cancer, systemic chemotherapy has been used extensively as a postoperative adjuvant therapy; one of the most popular regimens is cyclophosphamidum + epirubicin + 5-fluorouracil (CEA). However, a considerable percentage of patients experienced liver metastases during or after chemotherapy, irrespective of the administration of CEA, and the majority of them were not eligible for additional surgical resection (47).

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 (48). Elevated HbA_1c has been regarded as an independent risk factor for CAD in subjects with or without diabetes (49) (50). HbA1c values <7% were determined by the Diabetes problems and Control Trial (DCCT) to be the gold standard for glycemic control and to lower the risk of vascular problems (51). 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 (50). Thus drop in HbA_1c is associated with a drop in diabetes-related risk complications (48). Type 2 diabetic individuals are at a much higher threat of cardiovascular diseases than non-diabetic (9).

Thus the risk of cardiovascular events in diabetics can be abridged by improving glycemic control (52) (53). In the study that was presented, the patient group's levels of DPP-4 were considerably higher than those of the control group. When dipeptidyl peptidase 4 (DPP4) was first identified, it was thought to be a serine exopeptidase that broke amino-terminal dipeptides from polypeptides' N-terminus in the extracellular environment (54). Its primary function was thought to be the deactivation of two incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP), which cause abnormalities in glucose metabolism and decreased sensitivity to insulin (55). Sitagliptin, a DPP-4 inhibitor, is authorized for the management of diabetes. These medications lengthen the half-life of incretins by inhibiting the enzyme DPP-4 (56). Additionally, inhibition of DPP4 stops glucagon-like peptide-1 from inactivating, which promotes insulin production and

improved glycemic control. DPP4 inhibitors (DPP4i) are licenced and utilised in diabetes mellitus type 2 as monotherapy as well as in combination with metformin because of this mechanism of action (57).

Incretin-based medications called dipeptidylpeptidase-4 (DPP-4) inhibitors and glucagon-like peptide-1 (GLP-1) analogues are used as second or third line therapies for type 2 diabetes. Despite the positive benefits these medications have been linked to on hypoglycemia and body weight1, there have been worries that using them may raise the risk of developing specific neoplasms (58). One theory regarding the imbalance seen in the previously described RCTs is that weight loss brought on by GLP-1 analogues may improve breast mass detection and boost the uptake and precision of mammography (59). An antigen linked to tumour proliferation and the cell cycle is the Ki-67 protein. It is capable of assessing tumour cells' proliferative activity, which is linked to the emergence, progression, and prognosis of different types of tumours (60).

CONCLUSION

High levels of DPP-4, in individuals with diabetic breast cancer compared to a healthy subject might be used as a good screening marker for diabetic breast cancer. While no significant differences were observed in serum GLP-1 and Ki-67 protein levels between the patient groups and the control group. The finding of increased DPP-4 levels in the diabetic breast cancer group suggests that this enzyme could serve as a potential biomarker for early detection of breast cancer in the diabetic population. DPP-4 is involved in glucose homeostasis and has been linked to tumor growth and metastasis. Its upregulation in the context of concurrent diabetes and breast cancer warrants further investigation.

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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.

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