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Advances in Bioresearch

ORIGINAL ARTICLE

Assessment of Prediabetes, Diabetes, and Associated Complications using Biomarkers – A Cross-Sectional Study

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ABSTRACT
Biomarkers are crucial in clinical trials and patient treatment, as they help medical practitioners, researchers, and regulatory officials make scientifically sound decisions. However, in clinical studies, it can be challenging to determine how much weight to place on biomarker results versus clinical outcomes. To identify the influence of selected parameters in predicting the early onset of diabetes and associated complications in a particular population, a cross-sectional study was conducted for two years with 632 participants. Blood glucose, symptoms of glycemia, complications, and comorbidity conditions were correlated with stages of glycemia, and various biomarkers were correlated with stages of glycemia, complications, comorbidity, and drug therapy. The study revealed a strong correlation between the biomarkers and hyperglycaemic consequences. Moreover, many other biomarkers such as protein carbonyl and galectin-3 showed positive results, highlighting their importance in identifying the progression of hyperglycemia earlier.
***Keywords**: Biomarkers, Blood glucose, Complications, Comorbidity.*

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INTRODUCTION

Investment in finding new biomarkers for early detection is of the utmost importance; new and promising molecules have been emerging and achieving improved outcomes. Some biomarkers are associated with inflammation, adiposity, lipid oxidation, glycation, oxidative stress, and iron metabolism. This is relevant because the metabolic dysregulation observed in diabetic progression can have numerous etiologies. In accordance, it is anticipated that the answer may be found in a multiplex set of different biomarkers [1]. Prolonged elevated blood glucose concentration, the key clinical sign of diabetes, initiates an enhancement of reactive oxygen species derived from glucose autoxidation and glycosylation of proteins. Consequently, chronic oxidative stress overwhelms cellular endogenous antioxidant defenses and leads to acute and long-standing structural and functional changes in macromolecules resulting in impaired cellular functioning, cell death, and organ dysfunction. The oxidative stress provoked a chain of pathological events over time causing diabetic complications such as nephropathy, peripheral neuropathy, cardiomyopathy, retinopathy, hypertension, and liver disease. Under diabetic conditions, accompanying genome/epigenome and metabolite marker alterations may also affect glucose homeostasis, pancreatic β-cells, muscle, liver, and adipose tissue [2]. By providing deeper genetic/epigenetic insight into direct or indirect dietary effects, nutrigenomics offers a promising opportunity to improve the quality of life of diabetic patients. The relationship between diabetes treatment, HbA1c, lipid levels, cardiac outcomes, and survival very likely exceeds the complexity level that our current knowledge base allows us to comprehend. As more metabolic syndrome features are added to the equation, the level of complexity increases.

From the present study, our interpretation is that these biomarkers are the ones that, so far, are at a more advanced research stage and, thus, are more promising for clinical implementation. We believe that a biomarker multiplex is the most effective solution for better sensitivity and specificity in predicting progressors in T2D. Such an achievement would improve patients' health and decrease the national system's burden regarding diabetes.

MATERIAL AND METHODS

Study design: A prospective cross-sectional study

Patients were interviewed using a semi-structured pre-designed consent form with sections eliciting information on personal, demographic, clinical, lab reports, etc. Information was sought from the patient or his attendant/ relative.

The required data was obtained from the medical records databases that were searched to retrieve the records of patients who were diagnosed with prediabetes and diabetes and persistent progression in diabetes and who were under various diabetic complications and drug therapy.

Inclusion Criteria All patients with a provisional diagnosis of diabetes mellitus with type 2 diabetes mellitus, blood glucose levels between 70 to 300 mg/dL, in-patient, and outpatients are also to be included in the study.

Exclusion Criteria

Age below 20 years and above 70 were not included in the study. Type 1 diabetic patients, pancreatic abnormalities, pregnant women, breastfeeding women, and COVID-19-positive subjects were excluded from the study. Patients who had incomplete medical records or died from causes other than cancer were excluded from the study group.

Statistical Analysis:

Data were entered in an Excel spreadsheet for Windows and analyzed using GraphPad Prism 9. Data was distributed according to the parameters selected; Categorical variables were presented as percentages (%). The correlations of the selected factors were analyzed using multivariate analysis, correlation matrix with Pearson r correlation at 95% confidence interval and p-value was calculated using two-tailed. The considered p-value is at $p > 0.05$.

Methods:

Type 2 diabetes is a chronic metabolic disorder characterized by high blood sugar levels due to the body's inability to effectively use insulin. Biomarkers are measurable substances or indicators that can provide insights into a disease's presence, progression, and severity.

RESULTS AND DISCUSSION

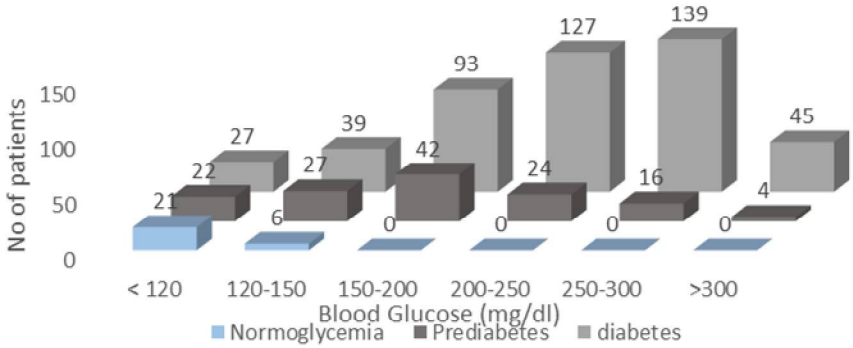

Figure 1: Distribution of subjects by Blood glucose Levels vs Gender

Blood Glucose Levels vs Stage of Glycemia

The study population was distributed between < 120mg/dL and >300 mg/dL. There was a strong correlation between blood glucose to prediabetic and diabetic conditions. Hyperglycemia is blood glucose greater than 125 mg/dL while fasting and greater than 180 mg/dL 2 hours postprandial. A patient has impaired glucose tolerance, or pre-diabetes, with fasting plasma glucose of 100 mg/dL to 125 mg/dL [3]. A patient is termed diabetic with fasting blood glucose of greater than 125 mg/dL. When hyperglycemia is left untreated, it can lead to many serious life-threatening complications that include damage to the eye, kidneys, nerves, heart, and peripheral vascular system. Thus, it is vital to manage hyperglycemia effectively and efficiently to prevent complications of the disease and improve patient outcomes [4]. The present study is 21.36% with 150-200mg/dL; 23.89% at 200-250mg/dL and 24.52% at 250-300mg/dL as shown in Table 4.12; indicating that possibilities and severe symptoms, risk factors associated complications and also need to assess the impact of other comorbidity conditions on elevated blood glucose levels and possibility of complications (figure 1).

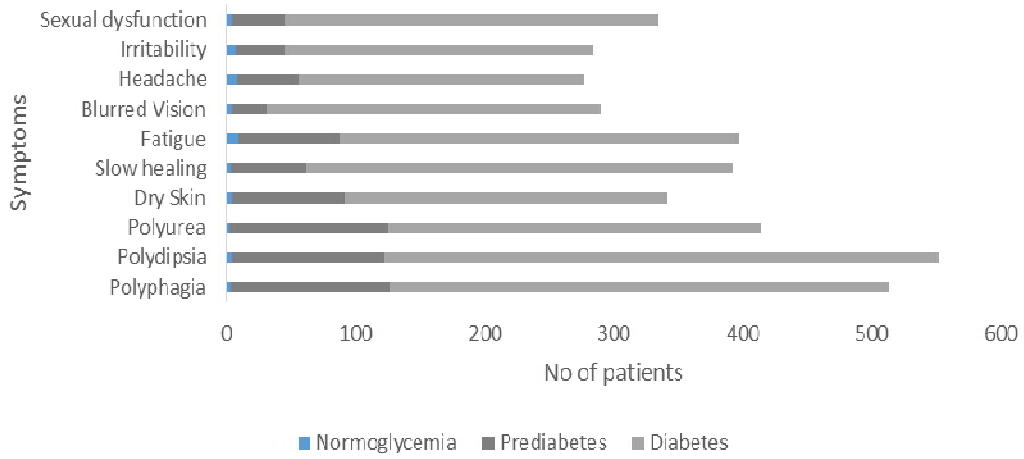

Figure 2: Distribution of subjects by Symptoms Vs stage of diabetes

Symptoms Vs Stage of Glycemia

The High-risk (3–5 risk factors) populations are more among. Polydipsia was the most happened symptom of the individual. Observed a strong correlation between glycaemic status (not with normoglycemia) and associated symptoms. Type 2 diabetes may lead to a variety of symptoms such as excessive thirst, frequent urination, fatigue, and burning feet. These symptoms diminish quality of life, impair functional status, and contribute to the psychological distress experienced by patients with diabetes. Many diabetes symptoms are linked through established pathophysiological mechanisms to inadequate short- or long-term glucose control or acute hypoglycemia [5]. Type 2 diabetes mellitus, usually shows (because there may not be any symptom) the following symptoms: frequent urination, especially in the evening (nocturia) – polyuria, polydipsia, polyphagia and intense hunger, weight loss, weakness / tiredness, lack of interest and concentration, vomiting and stomach pain, blurred vision, common infections and inflammation and wounds that are slow to heal and tingling at the extremities [6]. Diabetes symptoms and complications, smoking, and obesity (BMI) have all been associated with an increased risk of depression in previous studies. Patterns of reciprocal interactions between symptom severity, depression, and quality of life have been found in other chronic diseases such as asthma (Richardson LP., 2006). Polyphagia (82.91%); Polydipsia (89.08%); Polyurea (68.04%); Dry Skin, (55.06%); Slow healing (63.13%); Fatigue (62.82%); Blurred Vision (45.89%); Headache (43.83%); Irritability (44.94%) and Sexual dysfunction (53.16%) as presented in figure 2.

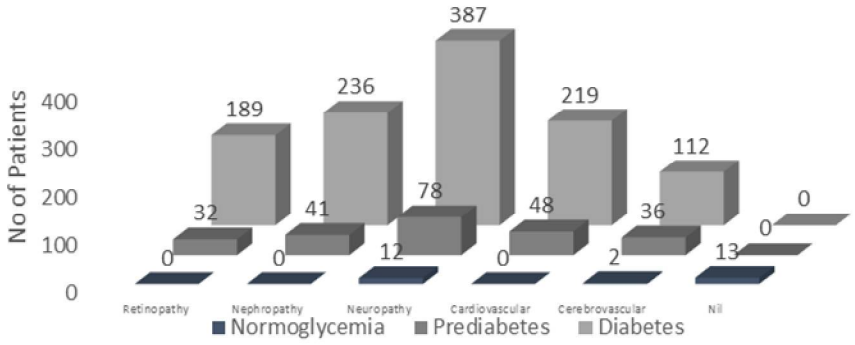

Figure 3: Distribution of subjects by Complications Vs stage of diabetes

Complications Vs Stage of Glycemia

The entire population was distributed according to the microvascular and macrovascular complications. The incidence of neuropathy was more compared to other complications. It was observed that a strong correlation between the stage of glycemia and diabetic complications. However, the pathogenesis of the long-term vascular complications associated with early- or late-onset type 2 diabetes is not well characterized and although the mechanisms for the development of complications may be similar, recent evidence suggests an accelerated course in people diagnosed with early-onset type 2 diabetes and the

biggest problem for diabetic patients is the long-term complications that accompany the disease. It was observed that the incidence of complications was found more in the present study followed by 34.97% with retinopathy; 43.83% with Nephropathy; 75.47% with Neuropathy; 42.25% with Cardiovascular complications and 23.73% with Cerebrovascular complications as shown in figure 3. In patients with type 2 diabetes previous prospective studies have shown an association between the degree of hyperglycemia and increased risk of microvascular complications, sensory neuropathy, myocardial infarction, stroke, macrovascular mortality, and all-cause mortality [7]. Previous studies reported that cardiovascular mortality is increased by 2 to 8 times in patients with type 2 diabetes mellitus, while 75% of deaths in these patients are attributable to underlying coronary artery disease [8].

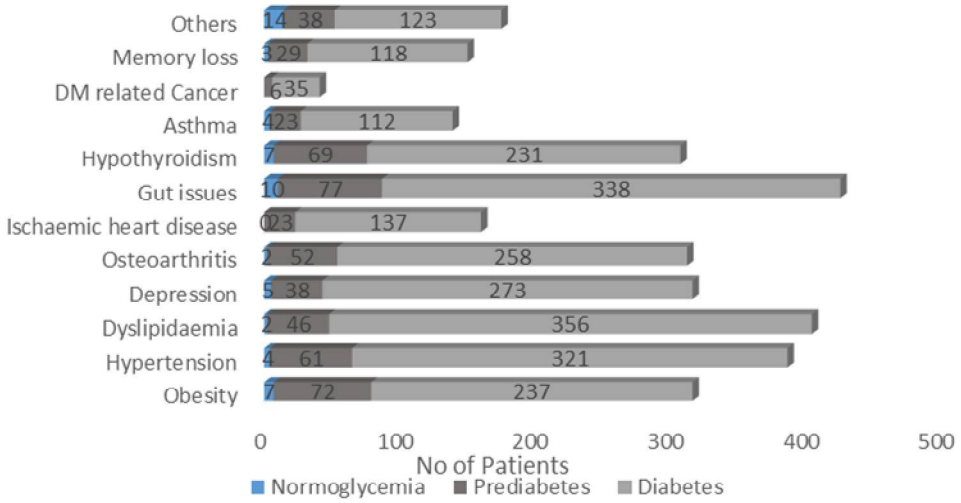

Figure 4: Distribution of subjects by comorbidity and stage of glycemia

Comorbidity Vs stage of glycemia

The entire population was separated according to the comorbidities reported. Gut issues and dyslipidemia are the most common possible comorbidities and the least with DM-related Cancers. There was a significant correlation is there between the glycemic stage and comorbidity conditions. Studies have demonstrated notable links between diabetes mellitus and a broad range of comorbidities, including cognitive decline, functional disability, affective disorders, obstructive sleep apnoea, and liver disease, and have refined our understanding of the association between diabetes mellitus and infection. Overall, one or more clinically diagnosed complications were present in 83.47% of the patients with type 2 diabetes included in this study. Due to similar risk factors, such as obesity, endothelial dysfunction, vascular inflammation, and dyslipidemia, people with T2DM have higher risks of cardiovascular complications, end-stage renal disease, and hypertension. However, individuals with T2DM have also been found to have higher risks of depression, thyroid gland diseases, and chronic obstructive pulmonary disease (COPD). People with multiple chronic conditions report several barriers to self-care such as physical limitations, lack of knowledge, financial constraints, logistics of obtaining care, and the need for social and emotional support. The present study population also distributed having multiple complications due to comorbidity conditions such as Obesity (50.79%), Hypertension (62.03%); Dyslipidaemia (65.98%); Depression (50.47%); Osteoarthritis (50.16%); Ischaemic heart disease (25.95%); Gut issues (68.20%); Hypothyroidism (49.37%); Asthma (21.99%); DM related Cancer (6.49%) and Memory loss (24.21%) as shown in figure 4

Table 1: Drug therapy and distribution according to the stage of glycemia

Comorbidities	Prediabetes (135)	Diabetes (470)	Total (605)
Biguanides	56 (41.48)	20 (4.25)	76 (12.56)
SU	70 (51.85)	31 (6.59)	101 (16.69)
Biguanides + SU	09 (6.66)	70 **(14.89)**	79 (13.05)
Biguanides+DPP-4i	0	44 (9.36)	44 (7.27)
Biguanides+SGLT2i	0	29 (6.17)	29 (4.79)
Biguanides + GLP-1RA	0	32 (6.80)	32 (5.28)

Biguanides + TZDs	0	47 (10)	47 (7.76)
Biguanides + Agi	0	20 (4.25)	20 (3.30)
Biguanides +SGLT2i +GLP-IRA	0	77 **(16.38)**	77 (12.72)
Biguanides +DPP-4i +Insulin	0	55 **(11.70)**	55 (9.09)
Biguanides + SGLT2i + GLP-IRA	0	45 (9.57)	45 (7.43)
P (two-tailed)	0.0002	<0.0001	
Significance	***	****	
r value	0.8769	0.9862	

Drug therapy Vs stage of glycemia

The study population is divided according to single therapy, dual, and triple therapy. The Biguanides +SGLT2i +GLP-IRA and Biguanides + SU are mostly prescribed patterns of therapy and there was a strong correlation between drug therapy and the stage of glycemia.

Table 2: To assess the correlation of selected biomarkers according to the stage of glycemia

Biomarkers	**Normoglycemia**	**Prediabetes**	**Diabetes**
FBG	98±4.67	119±3.68*	268±4.09*
HbA1c	4.23±1.5	5.32±0.89*	12.35±3.54*
Insulin	4.96 ± 1.99	5.87 ± 3.85*	10.52 ± 4.16*
HOMA-IR	2.73 ± 3.13	10.98 ± 2.17*	12.02 ± 3.22*
HOMA-β	102.0±4.34	109.9±3.33*	122.7±5.64*
HDL	38.10±1.14	39.17±2.12*	29.13±3.12*
LDL	126.08±2.17	133.34±1.89*	142.08±1.94*
Cholesterol	157.34±4.23	172.53±4.46*	210.71±5.89*
Triglycerides	163.13±1.27	168.90±1.46*	222.09±3.47*
AST	29.57±0.47	30.89±4.68*	33.90±2.02*
ALT	31.23±0.23	32.42±2.68*	38.09±5.33*
ALP	91.42±1.23	93.16±3.29*	105.42±6.09*
Bilirubin	0.34±0.08	0.41±0.09^{ns}	1.24±0.06*
Albumin	44.14 ± 6.62	43.17 ± 4.27*	48.29±4.44*
Protein	63.23±0.18	63.90±2.12*	68.16±5.09*
Uric acid	5.88±1.09	5.69±1.34 ^{ns}	6.74±1.69*
Protein Carbonylation	2.58±0.89	2.69±0.77 ^{ns}	4.12±1.07*
CRP	0.35±0.09	0.47±0.07 ^{ns}	1.21±0.06 *
Galactin - 3	09.23±1.74	17.23±3.07 $	28.34±2.43*
P (two-tailed)	0.0179	<0.0001	<0.0001
Significance	*	****	****
r value	0.6423	0.9109	0.9924

p>0.05 ^{ns}, p<0.05$^{\#}$, p<0.01$^{\$}$, p<0.001* Significance followed 2-way ANOVA, Dunnet's multiple comparison test with normoglycemia

Reference Values

FBG: Normal: 70 to 99 mg/dl; HaA1c: 3.8 - 6%; Insulin: 2-5 µU/mL; HOMA-IR: 0.45 – 1.28

ALT >45 U/L in men/>34 U/L in women, AST >35 U/L in men/ >31 U/L in women, ALP >129 U/L in men/>104 U/L, Bilirubin: 0.3-1.0 mg/dL; Albumin: 35–50 g/L; LDL: 0 – 155mg/dL; HDL:35-55mg/dL; triglycerides: 0 – 200mg/dL, total cholesterol: 100-200mg/dL; CRP:<1 mg/mL; Protein Carbonylation: nmol/mg of protein; Galactin-3 : ng/mL

Table 3: To assess the correlation of selected biomarkers according to complications

Biomarkers	**Retinopathy (221)**	**Nephropathy (277)**	**Neuropathy (477)**	**Cardiovascular (267)**	**Cerebrovascular (150)**	**Nil (13)**
FBG	278±6.87*	286±10.47*	257±9.08*	279±14.89*	271±12.38*	112±4.67
HbA1c	6.34±1.76*	7.89±1.99*	10.47±1.87*	9.44±2.14*	8.34±1.69*	3.89±2.12
Insulin	7.78±2.34*	5.67±1.89$^{\#}$	3.98±3.79*	6.78±3.33*	6.66±2.68*	4.96 ± 1.99
HOMA-IR	6.69±1.67*	10.98±2.12*	8.81±1.68*	7.89±3.01*	9.72±2.56*	2.73 ± 3.13
HOMA-β	108±3.87*	111±4.09*	105±3.23*	118±2.99*	107±2.56*	97±5.23
HDL	26.77±4.23*	30.12±2.47*	27.89±5.61*	28.18±3.63*	28.09±5.87*	36.24±3.09
LDL	143.09±3.56*	151.34±4.01*	146.56±2.86*	138.11±3.01*	134.12±2.79*	126.08±2.17
Cholesterol	210.12±2.34	216.46±2.09	185.11±3.11	193.09±3.09*	193.18±3.65*	157.34±4.2

	*	*	*			3
Triglycerides	222.47±2.23*	201.11±2.89*	170.75±3.98*	188.34±2.22*	177.70±2.32*	163.13±1.27
AST	32.14±1.23*	33.45±0.90*	30.09±1.67*	36.11±0.89*	34.65±1.45*	28.90±0.89
ALT	35.23±0.67*	34.12±0.98*	36.43±0.57*	37.09±1.79*	32.68±0.87*	30.59±0.70
ALP	103.34±2.12*	98.27±3.09*	99.09±2.56*	96.09± 2.98#	100.27±3.09*	95.23±2.23
Bilirubin	1.21±0.08#	1.09±0.09$	1.09±0.05$	0.79±0.07 ns	0.98±0.03 ns	0.43±0.04
Albumin	47.68±0.98*	46.09±0.87*	44.40±0.99*	43.56±0.56*	45.69±1.23*	40.19 ± 3.12
Protein	66.66±0.45*	65.27±0.78*	61.87±0.98#	63.98±1.09*	64.34±0.78*	60.99±0.44
Uric acid	6.09±0.77*	5.78±0.60$	5.98±0.23#	5.60±0.78ns	5.55±0.66 ns	5.16±0.91
Protein Carbonylation	3.56±0.23*	3.33±0.42*	2.89±0.31ns	3.18±0.56#	3.56±0.87*	2.34±0.77
CRP	0.91±0.06*	0.67±0.07*	0.55±0.07*	0.88±0.09*	0.71±0.07*	0.48±0.05
Galectin - 3	27.80±3.54*	21.09±4.32*	24.65±3.67*	23.98±2.76*	25.87±3.33*	08.78±1.56
P (two-tailed)	<0.0001	0.0006	0.0651	0.0008	<0.0001	<0.0001
Significance	****	***	ns	***	****	****
r value	0.7837	0.7152	0.4315	0.7024	0.8281	0.7837

p>0.05 ns, p<0.05#, p<0.01$, p<0.001* Significance followed 2-way ANOVA, Dunnet's multiple comparison test with nil complications

Table 4: To assess the correlation of selected biomarkers according to the Comorbidity Conditions more than 50% incidence

Biomarkers	Obesity	Hypertension	Dyslipidaemia	Gut issues	Depression	Hypothyroidism	Osteoarthritis	**Normoglycemia**
FBG	320±8.91*	259±10.54*	278±7.12*	269±14.32*	276±11.11*	291±8.23*	267±6.09*	98±4.67
HbA1c	9.45±2.46*	12.42±1.90*	9.36±2.13*	10.52±1.58*	6.89±2.01*	11.56±1.66*	7.90±1.89*	4.23±1.54
Insulin	7.87±1.87*	9.23 ±2.09*	6.67±1.56*	6.34±2.23*	5.76±1.32ns	6.12±3.33&	5.87±2.35ns	4.96 ± 1.99
HOMA-IR	8.42 ±1.67*	10.91 ±2.22*	9.34±1.46*	9.45±2.75*	4.07±2.22&	6.87±3.20*	4.56±2.54&	2.73 ± 3.13
HOMA-β	112.0±6.32*	118.71±3.98*	110.34±6.66*	108.90±5.98&	107.4±4.12&	106.34±5.12&	100.37±3.09#	102.0±4.34
HDL	27.56±2.34*	27.19±3.33*	29.87±2.87*	31.98±3.89*	35.87±2.59*	27.98±4.02*	33.33±2.22*	38.10±1.14
LDL	136.43±3.33*	149.41±2.32*	138.98±3.09*	137.89±2.22*	129.83±1.89*	133.45±1.72*	140.54±2.56*	126.08±2.17
Cholesterol	189.89±2.98*	198.78±4.87*	165.09±3.09*	179.87±2.87*	166.78±3.89*	172.09±4.44*	166.98±3.76*	157.34±4.23
Triglycerides	189.76±4.76*	202.45±5.42*	170.98±2.89*	181.09±3.76*	177.76±4.97*	198.45±5.09*	168.09±2.65*	163.13±1.27
AST	31.24±2.21*	32.76±2.43*	30.00±3.09ns	31.31±3.33*	31.09±2.09*	32.34±2.11*	30.99±1.88*	29.57±0.47
ALT	34.23±1.23*	36.09±2.33*	35.53±1.11*	33.69±1.45*	34.76±1.65*	33.20±0.23*	33.33±0.87*	31.23±0.23
ALP	98.89±0.45*	100.32±1.32*	99.35±0.87*	95.87±1.11*	95.53±0.45*	96.87±0.89*	94.87±1.23*	91.42±1.23
Bilirubin	0.74±0.04 ns	0.99±0.07&	0.90±0.06 ns	0.62±0.08 ns	0.76±0.09 ns	0.89±0.08 ns	0.56±0.07 ns	0.34±0.08
Albumin	44.14±4.56 ns	47.20±3.35*	43.87±2.67 ns	45.09±1.45*	46.81±2.09*	42.56±1.56*	44.88±3.01&	44.14 ± 6.62
Protein	67.12±0.45*	66.56±0.32*	64.72±0.47*	65.09±0.39*	62.56±0.88&	60.98±0.90*	63.98±1.09&	63.23±0.18
Uric acid	6.41±0.98 ns	6.74±0.78#	5.98±0.77 ns	5.78±0.67 ns	6.66±0.53#	6.72±0.69#	6.91±0.56*	5.88±1.09
Protein Carbonylation	3.61±0.77*	4.19±0.47*	3.33±0.74#	3.67±0.49*	3.98±0.56*	4.44±0.64*	2.98±0.45 ns	2.58±0.89
CRP	0.95±0.07 ns	1.03±0.06#	0.56±0.04 ns	0.65±0.09 ns	0.56±0.05 ns	0.44±0.07 ns	0.89±0.07 ns	0.35±0.09
Galectin - 3	25.56±3.12*	23.12±2.54*	2o.90±3.33*	19.66±2.09*	26.87±1.99*	24.45±2.43*	18.98±2.76*	8.19±1.23

P (two-tailed)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Significance	****	****	****	****	****	****	****	****
r value	0.8643	0.9327	0.8846	0.9041	0.8871	0.8831	0.8925	0.8643

$p>0.05^{ns}$, $p<0.05^{\#}$, $p<0.01^{\$}$, $p<0.001$* Significance followed 2-way ANOVA, Dunnet's multiple comparison test with normoglycemia

Table 5: To assess the correlation of selected biomarkers according to the drug therapy

Biomarkers	Biguanides (76)	SU (101)	B + SU (79)	B+SGLT2i +GLP-IRA (77)	B+DPP-4i +Insulin (55)
FBG	108±4.65*	128±8.96*	108±10.32*	98±4.68*	113±5.32*
HbA1c	4.09±1.76*	3.43±1.99*	4.32±1.87*	3.33±2.14*	3.23±1.69*
Insulin	5.98±2.56*	5.58±1.80#	6.43±4.19*	4.76±3.30*	4.32±2.48*
HOMA-IR	4.69±1.73*	6.09±2.45*	5.35±1.54*	3.01±3.61*	2.75±2.46*
HOMA-β	98±3.87*	102±3.33*	100±3.23*	97±2.61*	89±4.87*
HDL	30.75±4.08*	28.64±2.67*	33.10±5.45*	36.85±3.45*	35.53±4.43*
LDL	129.45±3.09*	132.86±2.67*	133.53±2.03*	130.42±2.78*	127.89±2.23*
Cholesterol	176.23±2.56*	170.43±2.12*	177.90±3.89*	153.68±2.75*	148.90±3.09*
Triglycerides	173.45±2.17*	180.98±2.22*	171.23±3.23*	167.07±2.25*	165.98±2.90*
AST	31.34±1.18*	30.98±0.34*	30.09±1.67*	28.99±0.63*	27.98±1.32*
ALT	33.08±0.88*	33.76±0.49*	32.76±0.44*	31.65±1.07*	30.87±0.67*
ALP	98.23±2.87*	97.09±3.12*	96.87±2.23*	95.56± 2.64#	94.67±3.14*
Bilirubin	0.70±0.03#	0.81±0.05$	0.56±0.05$	0.48±0.07 ns	0.45±0.07 ns
Albumin	47.12±0.98*	46.09±0.87*	44.09±0.23*	43.35±0.19*	41.90±1.78*
Protein	66.12±0.15*	64.23±0.23*	60.82±0.90#	64.12±1.17*	58.39±0.12*
Uric acid	5.23±0.25*	5.44±0.12$	5.35±0.56#	5.15±0.60ns	5.10±0.65ns
Protein Carbonylation	3.10±0.23*	2.76±0.42*	2.89±0.87ns	2.45±0.23#	2.24±0.54*
CRP	0.54±0.06*	0.60±0.07*	0.54±0.07*	0.56±0.07*	0.45±0.09*
Galactin - 3	13.34±4.65*	14.98±4.76*	13.86±4.65*	9.43±3.54*	11.23±3.26*
P (two-tailed)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Significance	****	****	****	****	****
r value	0.9947	0.9962	0.9951	0.9951	0.9974

$p>0.05^{ns}$, $p<0.05^{\#}$, $p<0.01^{\$}$, $p<0.001$* Significance followed 2-way ANOVA, Dunnet's multiple comparison test with normoglycemia

Biomarkers Vs stage of glycemia; complications; Comorbidity and Drug Therapy

The biomarkers play an important role in the various risk factors of diabetes. It was observed with glucose, HbA1c, insulin, lipid profile, liver enzymes, kidney markers, oxidative stress markers, and galectin 3. The statistical analysis revealed significant alterations with diabetes rather than prediabetes and showed a significant correlation between the stage of glycemia and levels of biomarkers. To identify the type of complications (microvascular & macrovascular) and their progression with biomarkers established and observed a strong correlation with retinopathy, nephropathy, cerebrovascular, and cardiovascular except neuropathy.

Table 1 explains the correlation between drug therapy and diabetes condition to assess the relationship between it. Sulfonylureas, a glucose-lowering drug, could be used as an adjunctive drug for metformin in the treatment of T2D [9]. In an analysis comparing two different drug combinations, namely sulfonylureas + metformin and DPP4-inhibitors + metformin, the addition of sulfonylureas to metformin demonstrated a higher risk of hypoglycemia and weight gain, indicating that DPP-4 inhibitors may be more suitable than sulfonylureas as adjunctive therapy to metformin for poorly controlled T2D patients [10]. In terms of blood glucose control, the combination therapy of sulfonylureas with metformin has shown a similar efficacy of glucose-lowering as other dual combinations, and there was no significant difference in the change of HbA1 among different combinations before and after the treatment.

In a 5-year follow-up trial, it was found that newly diagnosed T2D patients who received early combination therapy with metformin and vildagliptin, a DPP-4 inhibitor, had better long-term glycaemic control compared to those who only received early monotherapy with metformin. In addition, it has been reported that the combination of vildagliptin and metformin has a significant association with HbA1c reduction and body weight loss [11]. According to reports, the combination therapy of metformin and

sitagliptin has also shown a significant improvement in blood glucose levels in patients with T2D after hospital discharge. Moreover, a real-world study indicated that the initial combination therapy of metformin with sitagliptin exhibited a consistent and prolonged glycemic improvement for a period of up to 4 years [12], supporting the long-term effectiveness of this combination therapy. In another randomized controlled study, compared with the metformin-glimepiride combination (metformin sulphonyl ureas), the combination of metformin-gemigliptin (metformin-DPP-4 inhibitor) achieved more effective glycemic control in T2D patients without increasing the risk of hypoglycemia or weight gain, which may be related to improvements in gut microbiota [13]. Several studies indicate that combined therapy like dual therapy and trio therapies are shown to have better glycemic control over a long period than monotherapy of biguanides or SUs. Diagnosis of nephropathy was set by a urine albumin level >30µg/min for microalbuminuria, and >200µg/ min for macroalbuminuria, or estimated glomerular filtration rate (eGFR) >60mL/min/1.73. In India a high prevalence of deranged LFTs of about 71.2% and 70% respectively in individuals with T2DM. Our study also showed abnormal liver parameters with a relatively lower rate of 53% as compared to the above study. Moreover, the frequency of deranged LFTs reported, in the case of Indian diabetes is 50–70% [14].

Perry et al. found that an individual having high uric acid levels. Nakagawa et al. showed that uric acid is a significant and independent risk factor in predicting hyperinsulinemia. the association between high-level serum uric acid remains obscure. It is debatable whether serum uric acid is an independent risk factor for type 2 diabetes or it only emphasizes the association between other independent risk factors and type 2 diabetes [15].

The LDL-C often deposits excess cholesterol in the walls of blood vessels. An elevated level of serum LDL-C promotes atherosclerosis and consequently increases cardiovascular disease risk. The lower serum HDL-C level indicates less cholesterol clearance from the circulating blood into the liver. Low levels of HDL-C along with high levels of serum LDL-C predispose to premature atherosclerosis [16].

Human Galectin-3 is associated with the development of microvascular complications of diabetes mellitus like retinopathy, nephropathy, and neuropathy [17, 18]. The study identified role of galectin 3 in the progression of diabetic complications particularly in retinopathy at 27.80±3.54, Various findings suggest that Gal-3 accelerates the pathogenesis of metabolic diseases, by enhancing inflammatory infiltrates. Inhibition of Gal-3 can also prevent acute diabetic retinopathy. The mean serum galectin level of patients with macroalbuminuria was 30.1 ± 1.37 ng/ml, which was significantly higher than those with microalbuminuria having a mean galectin level of 22.85 ± 4.83 ng/ml. Galectin levels positively correlated with cholesterol and TG and negatively with HDL. Similarly, our results were also in close agreement with those of Vora *et al*., 2019 [19]. The role of galectin in diabetic retinopathy has been highlighted by Abu El-Asrar *et al*. observed that galectin-1 and vascular endothelial growth factor (VEGF) levels were significantly higher in vitreous samples from progressive diabetic retinopathy patients than in those from nondiabetic subjects [20]. They also found a significant positive correlation between the levels of galectin-1 and VEGF. Several prospective and cross-sectional studies found that serum and cerebrospinal fluid (CSF) Gal-3 levels were significantly higher in patients with AD than in age-matched healthy controls [21, 22]. Another clinical study further found that the expression of galactin-3 in the frontal lobe was increased in patients with AD, while Aβ oligomerization was enhanced [23, 24].

CONCLUSIONS

With advances in the treatment of diabetes mellitus and the associated increase in life expectancy, the face of complications of diabetes mellitus is changing. With the optimization of glycaemic control and the traditional complications of diabetes mellitus, we are instead beginning to see the deleterious effects of diabetes mellitus on the eye, liver, kidney, heart, brain, and other organs. Given the significant burden and risk of these emerging complications, future clinical and public health strategies should be updated accordingly. Awareness of emerging complications among primary care physicians in the first line of diabetes mellitus care needs to be increased and a place for screening for conditions such as depression, liver disease, and cancer should be considered in diabetes mellitus guidelines. Clinical care of older people with diabetes mellitus should focus on physical activity, especially strength-based activity, to reduce the risk of functional disability in the aging population. Continuous high-quality tracking of diabetes mellitus outcomes is essential to ensure we know where the burden lies. Due to the increasing burden of these emerging complications, the traditional treatment of diabetes mellitus may need to broaden its horizons. In general, combination therapy plays a pivotal role in the management of diabetes. Integrating the effectiveness of multiple drugs enables more comprehensive and effective control of blood glucose without increasing the risk of hypoglycemia or other serious adverse events. However, specific treatment regimens should be tailored to individual patients and implemented under the

guidance of healthcare professionals. We believe drug combination therapy will progress toward a more personalized approach. Considering the heterogeneity of diabetes, we hope to explore drug combination therapy based on finer subpopulations within diabetes, which will contribute to a more comprehensive understanding of individual responses to treatment, offering guidance for more effective clinical management. Increasing interest in biomarkers associated with DM goes back to its role in decreasing diabetes-related morbidity and mortality. Many studies have shown that the diagnosis of early-onset diabetes (eg, prediabetes) plays an important role in preventing its complications. Identification of new biomarkers can contribute to a better understanding of pathogenesis events involved in DM and can be powerful in detecting DM in early stages. Among various biomarkers, protein carbonyls, and Galectin 3 have emerged as interesting tools for detecting diabetes. These molecules play a critical role in cellular pathways involved in DM pathogenesis and may be a promising biomarker in identifying patients with diabetes mellitus.

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