Quantitative Assessment of Plasma Glucose, Hba1c, and Other Serum Biochemical Markers in Type 2 Diabetic Mellitus Patients from Andhra Pradesh, India

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Abstract

Type 2 diabetes mellitus (T2DM) is a major metabolic disorder characterized by persistent hyperglycaemia and frequently accompanied by dyslipidaemia and low-grade inflammation, which collectively accelerate vascular injury and cardiovascular risk. This study evaluated age-related alterations in key biochemical and inflammatory markers in T2DM. A total of 136 clinically diagnosed T2DM patients aged between 30 and 80 years were recruited from Guntur Government Hospital, and 136 apparently healthy, non-diabetic controls matched for age range were taken. Participants were stratified into five age groups (30–40, 41–50, 51–60, 61–70, and 71–80 years). Fasting blood samples were collected and analysed for plasma glucose using GOD-POD method, glycosylated haemoglobin using HbA1c, LDLc using direct enzymatic colorimetry, CRP using latex agglutination turbidimetry, homocysteine using enzyme cycling method, and ADA using enzymatic method. Statistical comparisons across age groups were performed using descriptive statistics, regression correlation, and one-way ANOVA (SPSS v10.0). Across all age strata, T2DM patients showed consistently higher values than controls for plasma glucose (144.42±10.52 to 170.24±8.03 mg/dL), HbA1c (6.66±0.59 to 10.53±0.88), LDLc (146.83±9.04 to 182.81±9.73 mg/dL), CRP (4.73±0.71 to 11.32±0.79 mg/dL), homocysteine (9.45±0.97 to 20.49±0.92 µmol/dL), and ADA (33.26±3.18 to 46.64±2.54 U/L). Most markers demonstrated significant age-associated increases with strong positive correlations in both groups (e.g., glucose, HbA1c, CRP, and ADA), showed a marked rise particularly in older age categories. Overall, the findings indicate that worsening glycaemic status in T2DM is accompanied by progressive inflammatory activation (CRP), atherogenic lipid burden (LDLc), elevated homocysteine, and increased ADA activity, supporting their combined utility as biomarkers for metabolic deterioration and vascular risk across ageing in T2DM.

Keywords:
Diabetes Mellitus, TCF7L2, KCNQ1, plasma glucose levels, homocysteine, adenosine deaminase

1. Introduction

Type 2 diabetes mellitus (T2DM) is considered the predominant type of diabetes, representing approximately 90-95% of all diabetes cases, and it is linked to irregularities in glucose, lipid, and protein metabolism. The symptoms of T2DM include adverse nitrogen status, hyperglycemia, hyperlipidemia, and glycosuria (1). Prolonged exposure to hyperglycemia can lead to malfunction and impairment of multiple organs, particularly the eyes, kidneys, nerves and cardiovascular system (2). The American diabetes Association states that cardiovascular disease (CVD) is responsible for 75-80% of morality in people with T2DM (3), T2DM anomalies are caused by a lack of insulin action on target tissues, which

can be related to insulin secretion deficiency, insulin action errors, or both. Increased insulin production initially compensates for insulin resistance; however, later on, insulin secretion is affected. Insulin production declines more quickly than insulin sensitivity when glucose tolerance and diabetes proceed from normal to impaired (4). Despite extensive research, many questions remain about the pathophysiology, categorization, symptoms, indicators, and assessment of T2DM. Assigning the pathogenesis of T2DM solely to hyperglycemia is inadequate; a thorough evaluation of the disorder is essential due to its complicated and multifaceted pathophysiology, marked by a high prevalence of vascular complications (5).

Based on recent studies, elevated blood sugar, inflammatory protein concentrations, and persistent low-grade inflammation all contribute significantly to the onset and progression of T2DM. Moreover, the long-term consequences of T2DM implicate protein glycation and HbA1c. Research has indicated that elevated HbA1c and total proteins are linked to lower albumin concentrations (6.). The etiology of T2DM also includes disruptions in lipid metabolism, leading to the buildup of modified lipid molecules in the bloodstream and tissues, s well as alterations in metabolic signaling pathways that govern insulin production from pancreatic beta cells (7.). Numerous investigations have demonstrated that blood lipid and lipoprotein abnormalities frequently coincide with diabetes. These changes raise concerns as they could potentially contribute to the increased risk of cardiovascular disease associated with diabetes (8.). The prevalent from of dyslipidemia in T2DM is characterized by higher triglycerides (TG), reduced levels of high-density lipoprotein cholesterol (HDLc), and a heightened occurrence of tiny low-density lipoprotein cholesterol (LDLc) (8.). Furthermore, various metabolic and inflammatory factors associated with the onset of T2DM, such as elevated blood glucose, adipokines, free fatty acid concentrations, and proinflammatory cytokines, may stimulate the synthesis of CRP (9.). Moreover, elevated CRP levels serve as a dependable indicator of vascular problems and the advancement of cardiovascular disease in diabetes individuals (10.). Moreover, research involving humans (11.) and animals (12.) has established a correlation between increased blood CRP levels, obesity, and the advancement of T2DM.

Recent studies indicate that plasma homocysteine (Hcy) levels, a sulphur-containing non-protein amino acid involves in methionine metabolism, are linked to the vascular problems of T2DM (13.). (14)found a correlation between increased homocysteine levels and insulin resistance in diabetic individuals. Numerous studies, including (15) and (16), have shown that increased homocysteine levels are predictive of morality and coronary events in patients with T2DM. Adenosine significantly influences insulin bioactivity and modulates its function across multiple tissues, including the liver, myocardium, white adipose tissue, and skeletal muscles, by enhancing insulin activity through various mechanisms, such as glucose transport, lipid synthesis, pyruvate dehydrogenase activity, and leucine oxidation (17); (18)(19) asserted that adenosine enhances the availability of GLUT4 on the cell surface, facilitating glucose transport by approximately 25%. Consequently, (20) propose that ADA is a crucial enzyme for regulating insulin biological action, but its clinical relevance in T2DM remains unverified. These inflammatory mediators may function as biomarkers for T2DM and are essential for identifying the early stages of T2DM, Hence, the present investigation aims to ascertain the amounts of plasma glucose, HbA1c, LDL, CRP, homocysteine, and adenosine deaminase in individuals with T2DM across different age groups.

2. Materials and Methods

The research study was approved by the Institutional Ethics Committee of Andhra University in Visakhapatnam. It involved 136 individuals aged 30 to 80 years who were diagnosed with T2DM and selected from the outpatient department of Guntur government hospital. The diagnosis was established in accordance with the American Diabetic Association (ADA) criteria, ensuring that participants met specific diagnostic, inclusion, and exclusion criteria. Following the description of the study's goal and objective, permission was obtained from all participants voluntarily. Subsequently, the patients were categorised into five distinct age groups, namely 30-40, 41-50, 51-60, 61-70, and 71-80. Upon receiving their permission, blood samples were collected, processed, and maintained for subsequent examination.

The study included cases, of male and female individuals from diverse racial and ethnic backgrounds, aged 30–80 years, diagnosed with T2DM and receiving oral hypoglycemic agents or insulin therapy for a maximum duration of two years. Only participants who provided informed consent were enrolled; when individuals were unable to provide consent, participation was permitted with consent from a legally authorized guardian. Individuals were excluded if they had type 1 diabetes mellitus, malignancy, genetic malformations, acute illness, or other endocrine disorders. Pregnant women, individuals with mental impairments, and those unwilling or unable to provide informed consent were also excluded. Additionally, T2DM patients with microvascular complications (diabetic neuropathy, nephropathy, retinopathy) or macrovascular complications (coronary artery disease, peripheral arterial disease, or stroke) were excluded from the study.

The control group consisted of 136 apparently healthy individuals recruited from participants attending a master health examination program, with no prior diagnosis of T2DM. Eligible individuals who met the control criteria were invited to participate in the study, and controls served as a baseline for comparison with T2DM patients to assess susceptibility to the disease. Male and female participants from diverse racial and ethnic backgrounds, aged 30–80 years and in good health, were included. Individuals were excluded if they declined to provide samples, were below 18 years of age, pregnant or breastfeeding, or had any coexisting medical conditions such as congestive heart failure, infections, fever, malignancy, nephritis, cirrhosis or autoimmune diseases.

2.1 Blood collection

Blood samples were obtained from specifically selected case participants and healthy control subjects of similar age and gender. After an overnight fast, 5 mL of blood was collected from each participant's peripheral vein. The collected blood was equally portioned into 2 EDTA vials and 1 sodium fluoride vial. The components have been carefully mixed and stored in a thermos flask with ice cubes. The blood samples were carefully transported to the laboratory. The blood samples from EDTA and fluoride vials were centrifuged at 3000 rpm for 15 minutes. The resulting serum was collected from all the tubes and kept at a temperature of -20ºC. The serum obtained from fluoride vials was used to determine glucose levels, and the blood pellet was used to estimate HbA1c. The serum taken from the EDTA tubes was used to measure the levels of LDL, CRP, Homocysteine, and Adenosine deaminase.

2.2 Evaluation of biochemical and serological markers associated with T2DM

The assessment of alteration in blood biochemistry triggered by diabetes was carried out by measuring plasma glucose and glycosylated haemoglobin (HbA1c). As well as, the effect of diabetes on serological markers was determined by the quantitative measurement of LDL, CRP, homocysteine (Hcy) and adenosine deaminase (ADA).

2.2.1 Estimation of plasma glucose: Glucose is the primary carbohydrate found in the bloodstream, and its oxidative breakdown provides energy to cells in the body. The determination of the amount of plasma glucose is carried out using the GOD-POD method. To determine the glucose concentration, 10 µL of serum from each blood sample was taken into separate test tubes, and to this, 1 mL of a glucose reagent containing GOD-POD was added. Simultaneously, blank and standard were maintained by taking 10 µL of distilled water and 100 mg/dL concentrated glucose solutions. The contents were mixed thoroughly and allowed to incubate for 10-15 minutes at a temperature of 37ºC. The colour intensity was quantified at a wavelength of 505 nm using spectrophotometry. The glucose concentration in the test sample was determined using the following formula:

$$Glucose(mg/dL) = \frac{Abof test - Abof blank}{Abof standard} X100$$

2.2.2 Estimation of glycosylated haemoglobin (HbA1c): To estimate glycolated haemoglobin, it is necessary to break down blood cells and prepare the haemoglobin fraction. The haemoglobin fraction was extracted following the method described by Adisa et al., (2004). Haemolysate was made using the method of hypotonic lysis. The red blood cells were washed three times with a 0.14 M NaCl solution. Then, one volume of the red blood cell suspension was lysed by adding two volumes of a 0.01 M phosphate buffer with a pH of 7.4 and 0.5 volume of CCl_4. The haemolysate was subsequently separated from the debris using centrifugation at 2300 rpm for 15 minutes at room temperature. Then the upper layer, containing a haemoglobin fraction, was removed and used to figure out the quantity of glycolated haemoglobin. In order to determine the level of glycosylated haemoglobin, 1 mL of prepared haemoglobin from each blood sample was taken in a separate test tube. Subsequently, 3 mL of concentrated H_2SO_4 was added to each test tube. The solution was left to cool for 30 minutes. Afterwards, 0.05 mL of an 80% phenol solution was added and incubated for 30 minutes at ambient temperature. Simultaneously, blank and standard were maintained by taking 1 mL of distilled water and 100 mmol concentrated standard glucose solution. The resulting colour was quantified at a wavelength of 480 nm using a spectrophotometer.

2.2.3 Estimation of LDLc : The LDLc comprises lipoproteins thar carry cholesterol to cells, frequently referred to as “bad cholesterol” due to its association with elevated risks for coronary heart disease, as well as its correlation with obesity, diabetes, and nephrosis. The determination of the serum LDLc concentration was carried out using the direct enzymatic colorimetric method (Atlas medical kit). Colorimetric determination of serum LDLc takes place in two steps. In the first step, cholesterol esters converted into cholesterol and fatty acids by cholesterol enzyme. Then the liberated cholesterol reacts with oxygen in presence of enzyme cholesterol oxidase and produce 4-cholestenone and H2O2. In the second step, the released H2O2 react with TOOS (N-ethyl-N-2-hydroxy-3-sulfopropyl-3-methylaniline) and 4-aminoantipyrine resulting in the production of a pigmented compound called quinonimine. This compound can be quantified using a spectrophotometer. The colour intensity generated is directly proportional to the LDLc content in the sample. To determine the LDLc concentration, 4 µL serum from each blood sample was taken into separate test tubes, and to this, 300 µL of R1 reagent was added which containing enzymes cholesterol esterase, cholesterol oxidase, TOOS, and catalase. The contents were mixed thoroughly and allowed to incubate for 5 minutes at a temperature of 37ºC. Simultaneously, blank and standard were maintained by taking 4 µL of phosphate buffer saline and standard provided in the kit. After incubation, 100 µL of R2 reagent which containing 4-aminoantipyrine and peroxidase was added. Then the reaction mixture was again incubated for 5 minutes at a temperature of 37ºC. Followed by incubation, the colour intensity was quantified at a wavelength of 600 nm using spectrophotometry. The LDLc concentration in the test sample was determined using the following formula:

$$LDLc(mg/dL) = \frac{Abofsample}{Abofstandard} XConc.ofStandard$$

2.2.4 Estimation of CRP : C-reactive protein (CRP) is a protein found in normal serum that surges following inflammation, malignant neoplasia, bacterial and viral infections, and most types of tissue damage. The serum CRP levels in both case and control subjects were determined using the latex agglutination method (Atlas medical kit), which is based on the idea that latex particles coated with anti-CRP agglutinate when they react with CRP-containing samples. The concentration of CRP in the sample is directly proportional to the agglutination of latex particles, which can be quantified using turbidimetry. Before conducting this experiment, working reagent was prepared according to the manufacturer’s instructions. The working reagent contains latex particles coated with goat anti-human CRP and diluent buffer which containing 20 mmol Tris buffer (pH 8.2) and 14.63 M sodium azide. In order to conduct this experiment, 5 µL of serum from each blood sample was taken into separate test tubes, and to this, 1000 µL of working reagent was added. Simultaneously, blank and standard were maintained by taking 5 µL of

distilled water and CRP-CAL standard provided in the kit. The contents were mixed thoroughly and measured the absorbance immediately (A1) at 540 nm against blank using spectrophotometry. Subsequently, the reaction mixture was incubated for 2 minutes and then measured the absorbance once again (A2). The CRP concentration in the test sample was determined using the following formula:

$$CRP(mg/L) = \frac{A2 - A1 of sample}{A2 - A1 of standard} X Conc.\ of Standard(mg/L)$$

2.2.5 Estimation of homocysteine (Hcy): The serum Hcy levels in both case and control subjects were determined using the enzyme circulation method (Assay Genie kit). To carry out this experiment, 39 µL of serum from each blood sample was taken into separate test tubes. Along with the test samples, blank and standards were maintained by adding 39 µL of homocysteine (0 µmol/L) and homocysteine (28 µmol/L) respectively provided in the kit. Then all the tubes were added with 720 µL of working solution-1, thoroughly mixed, and incubated at 37°C for 4 minutes. After incubation, 195 µL of working solution-2 was added, mixed thoroughly, and measure the absorbance at 0 min (A1) using spectrophotometer at 340 nm. Subsequently, the reaction mixture was incubated for 2 minutes and again measured the absorbance (A2). The Hcy concentration in the test sample was determined using the following formula:

$$Hcy(\mu mol/L) = \frac{\Delta A/min\ Sample - \Delta A/min\ Blank}{\Delta A/min\ Standard - \Delta A/min\ Blank} X Conc.\ of Standard(28\ \mu mol/L)$$

Where: $\Delta A/min\ (A1 - A2)/2\ min$

2.2.6 Estimation of adenosine deaminase (ADA): The serum ADA levels in both case and control subjects were measured using a commercially available kit (Elabscience). 10 µL of serum from each blood sample was transferred into separate test tubes. In addition to the test samples, blanks and standards were prepared by adding water and the standard included in the kit. Subsequently, each tube received 180 µl of working solution, was meticulously mixed, and incubated at 37°C for 7 minutes. Following incubation, the absorbance at 550 nm (A1) was determined using a spectrophotometer. Thereafter, the reaction mixture was incubated for 10 minutes, followed by a measurement of the absorbance (A2). A standard curve was constructed using the OD values of standards on the y-axis and their corresponding concentrations on the x-axis. The standard curve (y=ax+b) was plotted using Excel. The quantity of ADA required to produce on micromole of inosine from adenosine per minute at 37°C can be defined as one unit of ADA. The concentration of ADA in the test sample was ascertained using the following formula:

$$ADAactivity(U/L) = (A2 - A1 - b) \div aX1000 \div TXf$$

Where T = second incubation time
f = dilution factor of the sample

2.3 Statistical analysis

All the experiments were performed in triplicate, and the data were presented as the mean ± standard deviation. The results were analysed with descriptive statistics and regression correlation. Furthermore, the significance of the variation in means across the groups was determined using a one-way ANOVA. All the statistical analysis was performed by using SPSS Windows version 10.0.

3. Results

T2DM is characterised by two primary pathophysiological mechanisms: insulin resistance, particularly in skeletal muscle and liver, and impaired insulin production from the pancreas. Multiple variables related to metabolic regulation, glycemic variability, and serological elements play a crucial role in the onset of T2DM. Despite this, still not all pathogenic mechanisms were fully elucidated, as T2DM is a multifaceted condition arising from the interaction of genetic and environmental factors. This study evaluated the alterations in blood biochemistry caused by T2DM by quantifying plasma glucose, glycosylated haemoglobin (HbA1c), LDL cholesterol (LDLc), C-reactive protein (CRP), homocysteine (Hcy), and adenosine deaminase (ADA) levels.

3.1 Plasma glucose

Glucose levels are assessed to determine the glycaemic index of persons with T2DM. Table 1 presents the average plasma glucose levels for T2DM patients and healthy individuals across different age groups. The results indicated that average glucose levels in all test groups increased with age. The mean glucose levels in people with T2DM vary from 144.42±10.52 to 170.24±8.03 mg/dL with increasing age. In healthy individuals, glucose levels increase with age, often ranging from 84.10±8.23 to 101.61±5.69 mg/dL. The average glucose levels in people with T2DM across different age groups, such as 30-40, 41-49, 51-59, 61-69, and 70-80, were measured as 144.42±10.52, 160.11±7.99, 161.19±8.24, 163.78±8.16, and 170.24±8.03 mg/dL, respectively. The increase of glucose levels in people with T2DM was shown to be statistically significant ($P < 0.0001$) with increasing age groups. Table 2 displays the descriptive statistics of glucose levels in people with T2DM as age increases. The average glucose levels of healthy individuals increased with age, from 84.10±8.23 mg/dl in the youngest age group to 101.61±5.69 mg/dl in the eldest age group. The average glucose levels in healthy individuals across different age groups, such as 30-40, 41-49, 51-59, 61-69, and 70-80, were recorded as 84.10±8.23, 90.74±7.17, 92.29±8.29, 97.32±7.01, and 101.61±5.69 mg/dL, respectively. The association between age groups and glucose levels in healthy individuals was determined to be statistically significant ($P < 0.002$). Table 3 presents the descriptive statistics of glucose levels in healthy persons across varying ages. Moreover, strong correlations existed between glucose levels and increasing age groups in both T2DM patients and healthy controls, with coefficients of $r = 0.9176$ and $r = 0.9877$, respectively. The findings indicated a strong correlation between glucose levels and increasing age in both healthy individuals and patients with T2DM, as illustrated in the histogram (Figure1).

Table 1.Plasma glucose levels in T2DM patients and Healthy controls

S. No	Age Groups	Blood Glucose (mg/dl)	
		Healthy Controls	T2DMPatients
1	30-40	84.10±8.23	144.42±10.52
2	41-50	90.74±7.17	160.11±7.99
3	51-60	92.29±8.29	161.19±8.24
4	61-70	97.32±7.01	163.78±8.16
5	71-80	101.61±5.69	170.24±8.03

*Note:*Each value reported as mean ± standard deviation. $p< 0.05$ was considered as significant difference.

Figure 1
Histogram of plasma glucose levels in T2DM patients and Healthy controls

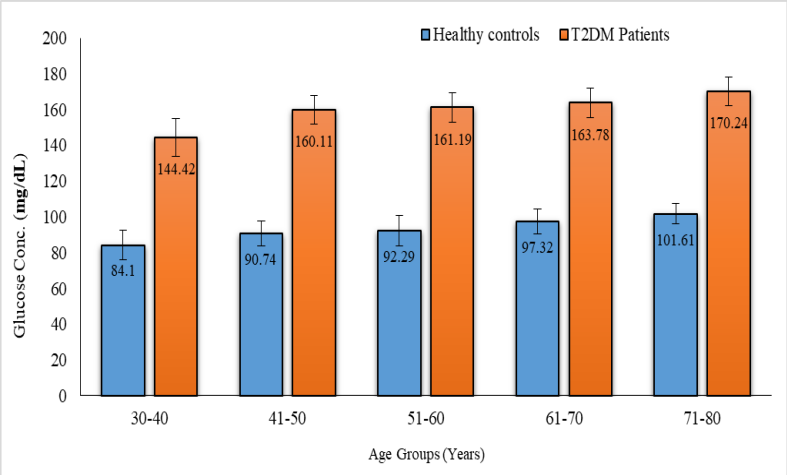

Table 2
Descriptive statistics of glucose levels in T2DM subjects with increase age

S. No.	Statistical parameter	Age groups				
		30-40	**41-50**	**51-60**	**61-70**	**71-80**
1	Mean	144.42	160.11	161.19	163.78	170.24
2	Standard deviation	10.52	7.99	8.24	8.16	8.03
3	Standard error	3.3	2.5	2.6	2.6	2.5
4	Variance	110.58	63.96	67.96	66.21	64.41
5	Kurtosis	-1.079	-1.061	3.631	-0.715	-1.249
6	Skewness	-0.123	-0.118	-1.327	-0.711	-0.241
7	Range	30.73	23	31.48	23.82	22.34
8	Minimum	127.88	148.58	141.66	149.62	158.88
9	Maximum	158.61	171.58	173.14	173.44	181.22
10	Confidence level (95%)	7.522	5.721	5.897	5.839	5.741

Table 3
Descriptive statistics of *plasma* glucose levels in healthy subjects with increase age

S. No.	Statistical parameter	Age groups				
		30-40	**41-50**	**51-60**	**61-70**	**71-80**
1	Mean	84.10	90.74	92.29	97.32	101.61
2	Standard deviation	8.23	7.17	8.29	7.01	5.69
3	Standard error	2.6	2.7	2.6	2.2	1.8
4	Variance	67.75	51.39	68.74	49.08	32.45
5	Kurtosis	-1.415	0.329	-1.313	-1.481	-0.824
6	Skewness	-0.153	-0.811	-0.160	-0.011	-0.252
7	Range	23.38	23.37	23.85	19.4	18.03
8	Minimum	71.24	76.44	79.83	87.86	91.68
9	Maximum	94.62	99.81	103.68	107.26	109.71
10	Confidence level (95%)	5.888	5.128	5.931	5.011	4.075

3.2 Glycosylated haemoglobin (HbA1c)

Table 4 presents the average HbA1c levels for people with T2DM and healthy individuals across various age groups. The data indicated that the average HbA1c values across all test groups increased with age. The mean HbA1c levels in people with T2DM vary from 6.66±0.59 to 10.53±0.88 mmol/mol with increasing age. In healthy individuals, HbA1c values rise with age, often ranging from 3.81±0.72 to 6.98±0.53 mmol/L. The average HbA1c levels in individuals with T2DM throughout age groups 30-40, 41-50, 51-60, 61-70, and 71-80 were recorded as 6.66±0.59, 7.25±0.56, 7.92±0.76, 9.48±0.89, and 10.53±0.88 mmol/L, respectively, and illustrated in Figure 2. The correlation between age groups and elevated HbA1c levels in T2DM patients was shown to be statistically significant (P=<0.0001). Table 5 presents the descriptive statistics of HbA1c values in patients with T2DM with increasing age. The average HbA1c values in healthy individuals escalated with age, recorded as 3.81±0.72, 4.21±0.78, 5.22±0.55, 6.19±0.57, and 6.98±0.53 mmol/L for each respective age cohort. The correlation between age groups and HbA1c levels in healthy individuals was shown to be statistically significant (P=<0.0001). Table 6 displays the descriptive statistics of HbA1c readings in healthy individuals across varying ages. Additionally, favourable associations were noted between HbA1c levels and advancing age groups in both T2DM patients and healthy controls, with correlation coefficients of r = 0.9925 and r = 0.9834, respectively. The results indicated a positive correlation between HbA1c levels and advancing age in both healthy individuals and patients with T2DM.

Table 4
HbA1c levels in T2DM patients and Healthy controls

S. No	Age Groups	HbA1c (mmol/L)	
		Healthy Controls	T2DM Patients
1	30-40	3.81±0.72	6.66±0.59
2	41-50	4.21±0.78	7.25±0.56
3	51-60	5.22±0.55	7.92±0.76
4	61-70	6.19±0.57	9.48±0.89
5	71-80	6.98±0.53	10.53±0.88

Note.Each value reported as mean ± standard deviation. p< 0.05 was considered as significant difference.

Figure 2
Histogram of mean HbA1c levels in T2DM patients and Healthy controls

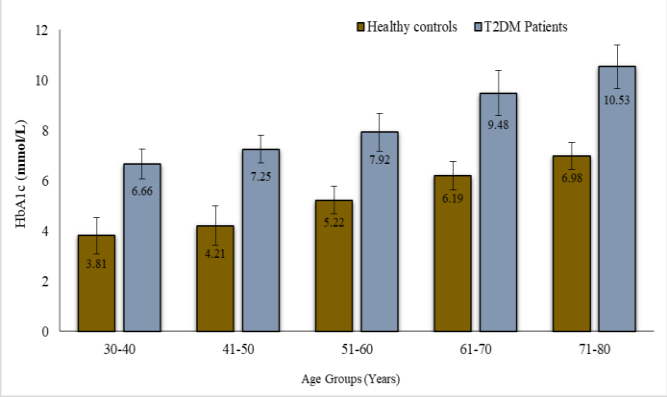

Table 5
Descriptive statistics of HbA1c in T2DM subjects with increase age

S. No.	Statistical parameter	Age groups				
		30-40	41-50	51-60	61-70	71-80
1	Mean	6.66	7.25	7.92	9.48	10.53
2	Standard deviation	0.59	0.56	0.76	0.89	0.88
3	Standard error	0.19	0.18	0.24	0.28	0.29
4	Variance	0.34	0.31	0.58	0.80	0.78
5	Kurtosis	-1.226	0.935	-0.920	-0.953	-0.829
6	Skewness	-0.032	0.886	0.347	-0.168	-0.59
7	Range	1.69	1.88	2.33	2.73	2.64
8	Minimum	5.87	6.54	6.91	7.99	8.98
9	Maximum	7.56	8.42	9.24	10.72	11.62
10	Confidence level (95%)	0.418	0.543	0.543	0.639	0.631

Table 6
Descriptive statistics of HbA1c levels in healthy subjects with increase age.

S. No.	Statistical parameter	Age groups				
		30-40	41-50	51-60	61-70	71-80
1	Mean	3.81	4.21	5.22	6.19	6.98
2	Standard deviation	0.72	0.78	0.55	0.57	0.53
3	Standard error	0.23	0.25	0.17	0.18	0.17
4	Variance	0.52	0.60	0.29	0.33	0.29
5	Kurtosis	-1.556	-1.396	-1.609	-1.418	-0.519
6	Skewness	0.031	-0.091	0.353	-0.494	-0.171
7	Range	1.85	2.2	1.5	1.5	1.7
8	Minimum	2.86	3.1	4.6	5.3	6.1
9	Maximum	4.71	5.3	6.1	6.8	7.8
10	Confidence level (95%)	0.517	0.555	0.392	0.411	0.383

3.3 Low-density lipoprotein cholesterol (LDLc)

The results of the mean serum LDLc levels in all age groups of T2DM patients and healthy subjects are shown in Table 7, and illustrated in Figure 3.These studies revealed that the average LDLc levels in all test groups increased with age. With respect to age, the average levels of LDLc in patients with T2DM vary from 146.83±9.04 to 183.81±9.73 mg/dL. Whereas, in healthy people, the levels of LDLc vary from 82.21±6.21 to 103.88±12.88 mg/dL. The mean LDLclevels in T2DM with increasing age groups such as 30-40, 41-50, 51-60, 61-70, and 71-80 were found to be 146.83±9.04, 151.62±9.55, 156.19±10.43, 161.33±6.93, and 182.81±9.73 mg/dL respectively. The LDLc content in T2DM patients significantly increased with age groups (P=<0.0014). Table 8 displays the descriptive statistics of LDLc content in T2DM patients with increasing age. Likewise, the average LDLc levels in healthy people with increasing age were found to be 82.21±6.21, 93.88±7.80, 96.83±7.57, 103.88±12.88, and 93.22±9.28 mg/dL respectively. The increase in LDLc levels among healthy individuals across different age groups was found to be statistically significant (P=<0.04). Table 9 displays the descriptive statistics of LDLc levels in healthy individuals as age increases. Moreover, these findings showed that LDLc levels had a strong positive connection (r = 0.9234) with increasing age in patients with T2DM. Conversely, LDLc levels had a moderate positive correlation (r = 0.646) with increasing age in healthy individuals.

Table 7
LDLclevels in T2DM patients and Healthy controls

S. No	Age Groups	LDLc(mg/dL)	
		Healthy Controls	T2DM Patients
1	30-40	82.21±6.21	146.83±9.04
2	41-50	93.88±7.80	151.62±9.55
3	51-60	96.83±7.57	156.19±10.43
4	61-70	103.88±12.88	161.33±6.93
5	71-80	93.22±9.28	182.81±9.73

Note.Each value reported as mean ± standard deviation. p< 0.05 was considered as significant difference.

Figure 3
Histogram of mean *LDLc* levels in *T2DM patients and Healthy controls*

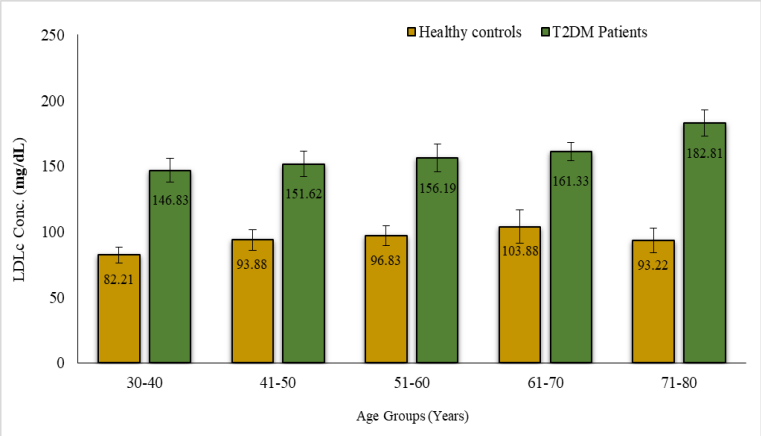

Table 8
Descriptive statistics of LDLcin T2DM subjects with increase age

S. No.	Statistical parameter	Age groups				
		30-40	41-50	51-60	61-70	71-80
1	Mean	146.83	151.62	156.19	161.33	182.81
2	Standard deviation	9.04	9.55	10.43	6.93	9.73
3	Standard error	2.86	3.02	3.29	2.19	3.08
4	Variance	81.68	91.18	108.75	48.06	94.63
5	Kurtosis	-0.458	-1.261	-0.529	0.283	0.081
6	Skewness	0.644	-0.194	-0.298	0.771	0.653
7	Range	27.27	26.89	34.3	23.01	32.44
8	Minimum	136.41	136.56	137.56	151.22	169.24
9	Maximum	163.68	163.45	171.86	174.23	201.68
10	Confidence level (95%)	6.465	6.831	7.460	4.959	6.959

Table 9
Descriptive statistics of HbA1c levels in healthy subjects with increase age

S. No.	Statistical parameter	Age groups				
		30-40	41-50	51-60	61-70	71-80
1	Mean	82.21	93.88	96.83	103.88	93.22
2	Standard deviation	6.21	7.80	7.57	12.88	9.28
3	Standard error	1.96	2.48	2.39	4.07	2.93
4	Variance	38.52	60.89	57.30	165.96	86.07
5	Kurtosis	-0.117	-0.554	0.014	-0.447	1.069
6	Skewness	0.422	-0.635	0.801	-0.137	-0.838
7	Range	20.16	24.06	23.89	39.94	32.06
8	Minimum	72.51	79.62	87.56	81.78	73.62
9	Maximum	92.67	103.68	111.45	121.72	105.68
10	Confidence level (95%)	4.439	5.582	5.415	9.215	6.637

3.4 C-reactive protein (CRP)

Table 10 and Figure 4, display the mean blood CRP levels across all age categories of T2DM patients and healthy individuals. The investigations indicated that the mean CRP levels in all test groups increased with age. The typical CRP values in patients with T2DM range from 4.73±0.71 to 11.32±0.79 mg/dL, depending on age. In healthy individuals, CRP levels range from 0.99±0.32 to 2.82±0.61 mg/dL. The average CRP values in individuals with T2DM throughout age groups 30-40, 41-50, 51-60, 61-70, and 71-80 were 4.73±0.71, 6.73±0.81, 7.92±0.63, 8.82±0.75, and 11.32±0.79 mg/dL, respectively. The CRP levels in T2DM patients significantly elevated with advancing age groups (P=<0.0001). Table 11 presents the descriptive data of CRP levels in T2DM patients as age increases. The average CRP levels in healthy individuals increased with age, recorded as 0.99±0.32, 1.53±0.43, 1.85±0.46, 2.56±0.43, and 2.82±0.61 mg/dL, respectively. The elevation of CRP levels in healthy persons across various age demographics was determined to be statistically significant (P=<0.0001). Table 12 presents the descriptive statistics of CRP levels in healthy persons as age progresses. Additionally, positive associations were noted between CRP levels and advancing age groups in both T2DM patients and healthy controls, with correlation coefficients of r = 0.9865 and r = 0.9916, respectively. The results indicated a strong correlation between CRP levels and advancing age in both healthy individuals and patients with T2DM.

Table 10
CRP levels in T2DM patients and Healthy controls

S. No	Age Groups	CRP(mg/dL)	
		Healthy Controls	T2DM Patients
1	30-40	0.99±0.32	4.73±0.71
2	41-50	1.53±0.43	6.73±0.81
3	51-60	1.85±0.46	7.92±0.63
4	61-70	2.56±0.43	8.82±0.75
5	71-80	2.82±0.61	11.32±0.79

Note.Each value reported as mean ± standard deviation. p< 0.05 was considered as significant difference.

Figure 4
Histogram of mean *CRP* levels in *T2DM patients and Healthy controls*

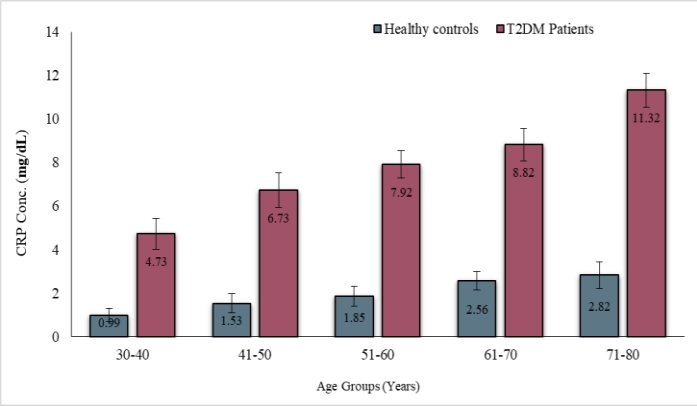

Table 11
Descriptive statistics of CRP in T2DM subjects with increase age

S. No.	Statistical parameter	Age groups				
		30-40	41-50	51-60	61-70	71-80
1	Mean	4.73	6.73	7.92	8.82	11.32
2	Standard deviation	0.71	0.81	0.63	0.75	0.79
3	Standard error	0.22	0.26	0.20	0.24	0.25
4	Variance	0.503	0.649	0.401	0.564	0.638
5	Kurtosis	-0.998	-0.927	-1.351	0.131	-0.050
6	Skewness	0.049	0.477	-0.082	0.691	-0.163
7	Range	2.04	2.34	1.79	2.48	2.74
8	Minimum	3.73	5.68	6.98	7.73	9.89
9	Maximum	5.77	8.02	8.77	10.21	12.63
10	Confidence level (95%)	0.507	0.577	0.453	0.537	0.572

Table 12
Descriptive statistics of CRP levels in healthy subjects with increase age

S. No.	Statistical parameter	Age groups				
		30-40	41-50	51-60	61-70	71-80
1	Mean	0.99	1.53	1.85	2.56	2.82
2	Standard deviation	0.32	0.43	0.46	0.43	0.61
3	Standard error	0.10	0.14	0.15	0.14	0.19
4	Variance	0.104	0.188	0.216	0.183	0.370
5	Kurtosis	-1.383	0.252	0.760	1.338	-0.790
6	Skewness	0.349	0.174	-0.309	-1.209	0.135
7	Range	0.94	1.45	1.59	1.46	1.93
8	Minimum	0.58	0.89	0.93	1.65	1.89
9	Maximum	1.52	2.34	2.52	3.11	3.82
10	Confidence level (95%)	0.231	0.309	0.332	0.306	0.435

3.5 Homocysteine

The mean homocysteine values of healthy individuals and patients with T2DM across various age categories are presented in Table 13 and Figure 5. According to this data, the average homocysteine values in the age groups of 30-40, 41-50, and 51-60 did not demonstrate a significant increase with age. However, in the age groups of 61-70 and 71-80, homocysteine levels increased substantially with age in all test groups. As individuals with T2DM age, their average homocysteine levels fluctuate between 9.45±0.97 and 20.49±0.92 µmol/dL. Homocysteine levels in healthy individuals typically range from 7.53±0.85 to 17.82±0.84 µmol/dL and increase with age. In the age groups of 30-40, 41-50, 51-60, 61-70, and 71-80, the mean homocysteine levels were 7.53±0.85, 7.98±1.15, 8.37±1.08, 15.67±0.81, and 17.82±0.84 µmol/dL of T2DM, respectively. Age groups were statistically significantly associated with an increase in homocysteine content in T2DM patients (P=<0.0019). Table 14 illustrates the descriptive statistics of homocysteine values in patients with T2DM and advancing age. The mean homocysteine values in healthy individuals increased with age, with values of 9.45±0.97, 10.3±0.76, 11.04±0.89, 18.75±1.11, and 20.49±0.92 µmol/dL for each corresponding age group. A statistically significant association was demonstrated between the homocysteine content of healthy participants and their age groups (P=<0.00098). The descriptive statistics of homocysteine values in healthy individuals as they age are presented in Table 15. Additionally, positive correlations were observed between the homocysteine levels and increasing age groups in both the T2DM patients and healthy controls, with correlation coefficients of r = 0.9297 and r = 0.9159, respectively. The results indicated that there was a substantial increase in homocysteine content in relation to T2DM, whereas age did not have a significant impact.

Table 13
Homocysteine levels in T2DM patients and Healthy controls

S. No	Age Groups	Homocysteine (µmol/dL)	
		Healthy Controls	T2DM Patients
1	30-40	7.53±0.85	9.45±0.97
2	41-50	7.98±1.15	10.3±0.76
3	51-60	8.37±1.08	11.04±0.89
4	61-70	15.67±0.81	18.75±1.11
5	71-80	17.82±0.84	20.49±0.92

Note. Each value reported as mean ± standard deviation. p< 0.05 was considered as significant difference.

Figure 5
Histogram of mean *homocysteine* levels in *T2DM patients and Healthy controls*

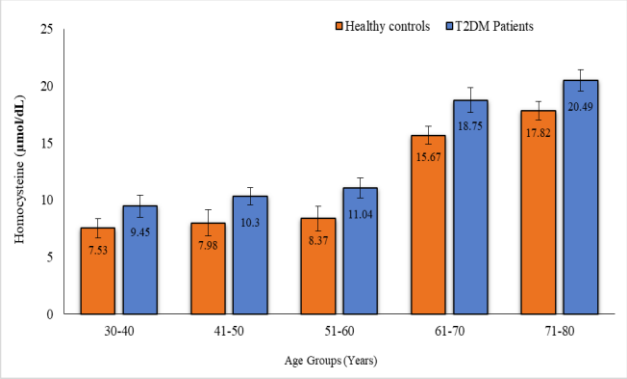

Table 14
Descriptive statistics of homocysteine in T2DM subjects with increase age

S. No.	Statistical parameter	Age groups				
		30-40	41-50	51-60	61-70	71-80
1	Mean	9.45	10.3	11.04	18.75	20.49
2	Standard deviation	0.97	0.76	0.89	1.11	0.92
3	Standard error	0.31	0.24	0.28	0.35	0.29
4	Variance	0.939	0.578	0.799	1.241	0.847
5	Kurtosis	0.318	-0.826	-1.647	-1.503	-0.699
6	Skewness	-0.336	0.344	0.034	-0.079	-0.107
7	Range	3.39	2.34	2.36	2.99	2.93
8	Minimum	7.62	9.24	9.86	17.22	18.92
9	Maximum	11.01	11.58	12.22	20.21	21.85
10	Confidence level (95%)	0.693	0.543	0.639	0.797	0.659

Table 15
Descriptive statistics of homocysteine levels in healthy subjects with increase age

S. No.	Statistical parameter	Age groups				
		30-40	41-50	51-60	61-70	71-80
1	Mean	7.53	7.98	8.37	15.67	17.82
2	Standard deviation	0.85	1.15	1.08	0.81	0.84
3	Standard error	0.27	0.36	0.34	0.26	0.27
4	Variance	0.721	1.313	1.156	0.657	0.712
5	Kurtosis	-0.560	-1.013	-0.519	-0.513	-1.394
6	Skewness	-0.716	-0.007	-0.199	-0.299	0.438
7	Range	2.51	3.61	3.37	2.61	2.28
8	Minimum	6.11	6.24	6.49	14.23	16.87
9	Maximum	8.62	9.85	9.86	16.84	19.15
10	Confidence level (95%)	0.607	0.819	0.769	0.579	0.604

3.6 Adenosine deaminase (ADA)

Table 16 and Figure 6 display the mean ADA values among patients with T2DM and healthy individuals across different age groups. Based on these data, it was shown that the average ADA values in all test groups increased with age except 71-80 age group in T2DM which exhibit decreased ADA level than 61-70 age group. With respect to age, the average levels of ADA in patients with T2DM vary from 33.26±3.81 to 46.64±2.54 U/L. Whereas, in healthy people, the levels of ADA vary from 8.37±0.99 to 20.09±1.38 U/L. The mean ADA levels in T2DM with increasing age groups such as 30-40, 41-50, 51-60, 61-70, and 71-80 were found to be 33.26±3.18, 38.66±1.71, 42.93±3.29, 46.64±2.54, and 45.33±3.05 U/L respectively. The association between age groups and an increase in ADA content in T2DM patients was shown to be statistically significant (P=<0.0001). The descriptive statistics of ADA values in patients

with T2DM and increasing age are presented in Table 17. Besides, the mean ADA values in healthy persons increased with age, measuring 8.37±0.99, 12.32±1.41, 12.85±0.78, 14.26±0.93, and 20.09±1.38 U/Lfor each corresponding age group. The association between age groups and ADA content in healthy participants was shown to be statistically significant (P=<0.00037). Table 18 presents the descriptive statistics of ADA values in healthy persons with increasing age. Furthermore, there were positive correlations observed between the ADA levels and increasing age groups in both the T2DM patients and healthy controls, with correlation coefficients of r = 0.9311 and r = 0.9452, respectively. The results showed that there a significant increase in ADA content with respect to T2DM and age.

Table 16
Adenosine deaminase levels in T2DM patients and Healthy controls

S. No	Age Groups	ADA (U/L)	
		Healthy Controls	T2DM Patients
1	30-40	8.37±0.99	33.26±3.18
2	41-50	12.32±1.41	38.66±1.71
3	51-60	12.85±0.78	42.93±3.29
4	61-70	14.26±0.93	46.64±2.54
5	71-80	20.09±1.38	45.33±3.05

Note. Each value reported as mean ± standard deviation. p< 0.05 was considered as significant difference.

Figure 6
Histogram of mean *ADA* levels in *T2DM patients and Healthy controls*

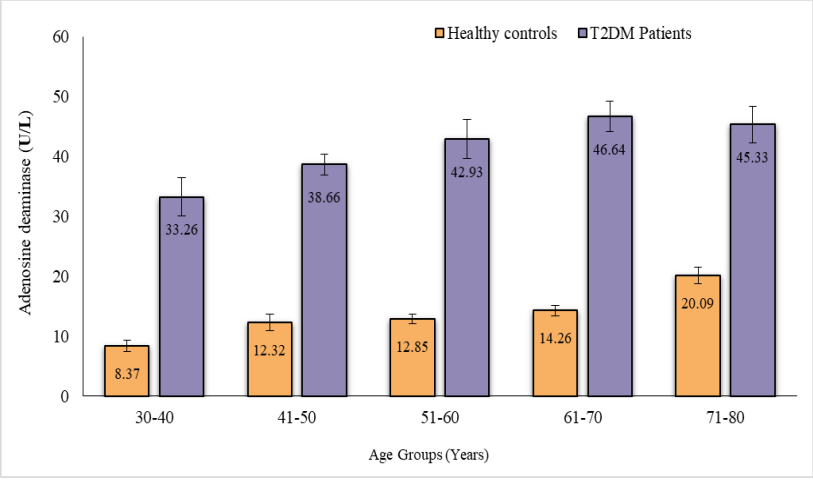

Table 17
Descriptive statistics of ADA in T2DM subjects with increase age

S. No.	Statistical parameter	Age groups				
		30-40	41-50	51-60	61-70	71-80
1	Mean	33.26	38.66	42.93	46.64	45.33
2	Standard deviation	3.18	1.71	3.29	2.54	3.05
3	Standard error	1.01	0.54	1.04	0.80	0.97
4	Variance	10.13	2.91	10.85	6.43	9.31
5	Kurtosis	-1.355	-0.396	-1.311	-0.169	-0.569

6	Skewness	-0.133	0.271	-0.171	-0.786	-0.454
7	Range	9.3	5.61	9.88	7.92	9.44
8	Minimum	28.56	36.11	37.68	41.8	39.82
9	Maximum	37.86	41.72	47.56	49.72	49.26
10	Confidence level (95%)	2.277	1.221	2.356	1.813	2.183

Table 18
Descriptive statistics of ADA levels in healthy subjects with increase age

S. No.	Statistical parameter	Age groups				
		30-40	41-50	51-60	61-70	71-80
1	Mean	8.37	12.32	12.85	14.26	20.09
2	Standard deviation	0.99	1.41	0.78	0.93	1.38
3	Standard error	0.31	0.44	0.25	0.29	0.44
4	Variance	0.989	1.977	0.615	0.859	1.898
5	Kurtosis	-1.414	-0.689	-0.844	-1.583	-1.153
6	Skewness	-0.179	-0.367	-0.014	-0.002	-0.435
7	Range	2.94	4.4	2.44	2.7	3.83
8	Minimum	6.87	9.82	11.68	12.92	17.89
9	Maximum	9.81	14.22	14.12	15.62	21.72
10	Confidence level (95%)	0.712	1.006	0.561	0.663	0.986

4. Discussion

T2DM is a widespread and increasing worldwide health concern that can severely disrupt healthcare systems in many different countries. It is a group of chronic conditions characterised by insufficient insulin production, cellular resistance to insulin, or both, resulting in elevated blood glucose levels and other related metabolic disturbances. (21) indicate that the diabetes is associated with considerable challenges impacting multiple organ systems, including the eyes, kidneys, heart, and blood vessels. These repercussions may significantly impair the patient's quality of life and decrease their survivality. According to (22) the prevailing method for diagnosing and monitoring diabetes entails the evaluation of plasma glucose levels. The present study sought to evaluate the relationship between plasma glucose, HbA1c, LDLc, CRP, homocysteine, and ADA levels in individuals with T2DM and those without the condition, focusing on age as a variable.

4.1 Plasma glucose

The results of the present investigation demonstrated a significant elevation in plasma glucose levels correlated with advancing age in both individuals with T2DM and those without the disorder. Moreover, people with T2DM demonstrated elevated plasma glucose levels. Multiple causes are believed to affect the observed elevation in plasma glucose levels with advancing age, and a proposal suggests that age may influence the glycaemic index of specific dietary products. The plasma glucose levels in individuals with T2DM ranged from 144.42±10.52 to 170.24±8.03 mg/dL, correlating with increasing age. In healthy individuals, glucose levels rise with age, typically ranging from 84.10±8.23 to 101.61±5.69 mg/dL. Furthermore, both T2DM patients and healthy controls showed significant associations between glucose levels and increasing age groups, with coefficients of r = 0.9176 and r = 0.9877 respectively.

The results of the present investigation align with the findings of (23) who found that plasma glucose levels were lower in the younger age group (20-29 years) and higher in the older age group (>69

years) of the Indian population. (24) demonstrated a positive relationship between age and plasma glucose levels in the Hong Kong Chinese population. Furthermore, they identified a positive correlation between age and random and fasting plasma glucose levels, showing a rise of 0.15 mmol/L for every ten years of age. Additionally, a 2-hour postprandial plasma glucose measurement indicated an elevation of 0.26 mmol/L. According to WHO, (2007) the prevalence of diabetes increases from roughly 6% in individuals 45-65 years old to 12% in those 65 and above. In non-diabetic adults, fasting plasma glucose (FPG) levels increased by 0.7-1.1 mg/dL for each decade of age, whereas 2-hour glucose levels increased by 5.6-6.6 mg/dL every decade. Numerous demographic studies such as (25) have documented this pattern. (26) indicated that the older cohort exhibited the lowest diagnostic rate with the FPG levels (45.5%) and the greatest diagnostic rate with the 2-hr plasma glucose (84.6%). The results were mostly attributed to the increased incidence of isolated post-challenge hyperglycemia in older individuals compared to the younger cohort (28.8% vs 9.2%). (27)observed that fasting blood glucose and HbA1c levels were significantly ($P < 0.05$) elevated in the age group of 57-74 years compared to those aged 40-56 years.

Glucose metabolism is essential for appropriate physiological function. The liver is a principle metabolic regulating organ, supplying 90-95% of circulating glucose during the postabsorptive stage, when the body ceases nutrition absorption from the gastrointestinal tract. The brain uses approximately 50% glucose during the post-absorptive phase, while skeletal muscle consumes around 15%. In healthy individuals, skeletal muscle exhibits high sensitivity to insulin, capable of enhancing glucose absorption by up to 85% in response to rapid elevations in plasma glucose levels (28). Insulin production from β cells is closely linked to glucose accessibility, enabling precise regulation of blood sugar concentrations within a normal range. As individuals age increases, some experience a gradual decline in their capacity to regulate glucose levels as effectively as they had in their youth (29.). The reduction in glucose tolerance from youth (17-39 years) to middle age (40-59 years) is universally attributed to the secondary effects of adiposity and physical fitness. Nonetheless, alterations in glucose tolerance observed between the ages of 60 and 92 were substantial and remained unaccounted for even after including body structure and physical activity in the analysis (30).

4.2 Glycosylated haemoglobin (HbA1c)

HbA1c levels are essential for the diagnosis, evaluation, and management of T2DM. The American Diabetes Association (ADA) standards stipulate that diabetes can be diagnosed through the assessment of glucose levels, an oral glucose tolerance test, or the measurement of HbA1c values (ADA, 2021). The concentration of HbA1c in red blood cells is directly proportional to plasma glucose levels, as it undergoes glycation through a non-enzymatic reaction (31). HbA1c is essential in the management of diabetic patients, serving as a marker to evaluate metabolic position, evaluate the risk of diabetes problems, and guide treatment decisions (32). Research indicates that metabolic regulation in non-diabetic persons alters with age, resulting in elevated HbA1c levels because of physiological processes (33). Recent research has revealed that HbA1c variability is an individual risk factor for T2DM complications in people with the disease (34). It may even be a more accurate indicator of glycaemic management than the mean HbA1c in T2DM (35). Moreover, (36) proposed that glycaemic variability is recognised as a marker of impaired glycaemic control, potentially functioning as a predictor for diabetic complications. Thus, assessing the variation of HbA1c levels over a prolonged duration may functions as a reliable indicator for the probability of diabetes onset. An investigation by (37) showed that the longer the condition has been present, the more carbohydrates are linked to the HbA1c.

The present investigation examined the relationship between HbA1c levels and age in patients with T2DM and healthy subjects. The results demonstrate that HbA1c levels were significantly higher in T2DM patients than in healthy individuals. In healthy individuals, HbA1c values rise with age, often ranging from 3.81±0.72 to 6.98±0.53 mmol/L. The typical HbA1c readings in people with T2DM range from 6.66±0.59 to 10.53±0.88 mmol/mol with advancing age. Additionally, positive associations were noted between HbA1c levels and advancing age groups in both T2DM patients and healthy controls, with correlation coefficients of $r = 0.9925$ and $r = 0.9834$, respectively. The present finding of our study exhibit

colinearity with the research conducted by (27). which indicated that HbA1c levels increased in the 57-74 years age group compared to the 40-56 years group. (38) demonstrated a favourable connection between age and HbA1c levels in persons without a genetic predisposition to diabetes. Furthermore, they noted that glycohemoglobin levels were affected by age irrespective of the diabetes type. (33) discovered that HbA1c levels gradually increase with age, resulting in a 0.074% elevation in HbA1c per decade. (39) suggested that the increase in haemoglobin glycation, attribute to the progression of age and leading to diabetic complications, is not solely influenced by blood glucose levels. Consequently, it may be determined that the highest permissible HbA1c level in older adults without glucose homeostasis concerns is 6.60%. This discovery reinforces the current data that prolonged variations in blood glucose levels are significantly associated with an increased risk of T2DM.

Numerous investigations, such as those by (40) and (41) have demonstrated that the variation in HbA1c values within an individual is negligible. Nonetheless, there is evidence of considerable discrepancies in HbA1c values across individuals that are not associated with their glycaemic status. This indicates that those with low HbA1c levels are termed high glycators. (42) discovered that the increasing utilisation of HbA1c for the long-term management of glucose in diabetes patients demonstrates a substantial association between the levels of HbA1c and the possibility of adverse effects. A thorough understanding of the relationship between plasma glucose and HbA1c is essential for patients and healthcare providers to set appropriate daily blood glucose testing goals with the goal of achieving predetermined HbA1c targets. (43) asserts that the subsequent hyperglycemia leads to increased protein glycation, resulting in elevated levels of glycated hemoglobin in the serum. (44) have demonstrated that glucose and Hba1c levels increase as a result of ageing-related declines in muscle mass, insulin sensitivity, insulin receptor activity, pancreatic β-cell function, and glucose intake. Moreover,(45) found that variations in HbA1c levels can increases the quantity of a marker indicative of systemic inflammation, which is associated with vascular damage. (36) asserted that HbA1c variability significantly influences the outcomes of diabetes through cellular metabolic memory, which differs from short-term glycaemic fluctuations.

4.3 Low-density lipoprotein cholesterol (LDLc)

LDL cholesterol serves as the primary lipid indicator for assessing risk for cardiovascular disease and is the essential treatment target in individuals with diabetes (46). In diabetic, abnormal lipid profiles are more prevalent, and they worsen when glycaemic management is inadequate. Elevated LDL cholesterol and reduced HDL cholesterol may result from obesity, excessive caloric consumption, and insufficient physical activity in individuals with T2DM (47). Consequently, assessing the lipid profile is essential to examine the impact of diabetes on lipid metabolism, particularly on HDL and LDL cholesterol (48). The NCEP, (2002) states that LDL cholesterol levels in individuals with diabetes do not exceed those in age, sex, and body weight-matched individuals without diabetes. The predominant LDL cholesterol concentration in people with diabetes is classified as “borderline high”, ranging from 130 to 159 mg/dL.

The current findings indicate that the mean blood LDLc values in all test groups escalated with age. The average levels of LDLc in patients with T2DM range from 146.83±9.04 to 183.81±9.73 mg/dL, depending on age. In healthy individuals, LDL cholesterol levels range from 82.21±6.21 to 103.88±12.88 mg/dL. Furthermore, these data indicated a robust positive correlation ($r = 0.9234$) between LDLc levels and advancing age in patients with T2DM. In contrast, LDLc levels exhibited a moderate positive connection ($r = 0.646$) with advancing age in healthy people. The findings of the current investigation corroborate with the earlier discoveries of (49) who observed elevated LDL and reduced HDL cholesterol levels in diabetes patients. Similarly, (50)found that LDL cholesterol levels were elevated, whereas HDL cholesterol levels were reduced in people with T2DM compared to control. (51) indicated that LDL cholesterol is considerably higher ($P<0.003$) in patients with type 1 diabetes compared to the control group. However, a minor link existed between LDL cholesterol levels in patients with T2DM. The present investigation’s discoveries align with the previously presented findings.

In individuals with T2DM, the critical involvement of diabetic dyslipidaemia, particularly low-density lipoprotein cholesterol (LDLc), in the progression of CVD and serious cardiovascular diseases, as well as hereditary risk factor and hyperglycemia (52). (53) found that diabetic patients tend to have more atherogenic LDLc particles distributions compared to healthy individuals, likely due to the smaller size of LDLc particle distributions compared to healthy individual, likely due to the smaller size of LDLc particles. Additionally, 30% of insulin-stimulated consumption of glucose occurs in the liver, and insulin resistance impairs glucose output and fatty acid metabolism, which raises the triglyceride levels and causes the liver to secrete more VLDL (54). The elevation of lipid from several sources, including circulating free fatty acids, the dissolution of triglyceride rich lipoprotein, and denovo lipogenesis, facilitates the posttranslational stabilisation of apoB and promotes the formation and release of VLDL particles. This result in the production of FFA and VLDL, which transport energy from the liver to the adipose tissue (55).(56) indicated that insulin release inhibits VLDL production to regulate plasma triglyceride levels. When people with T2DM have smaller dense LDLc, they also have more apolipoprotein B, which tends to move into the artery wall (57).

4.4 C-reactive protein

C-reactive protein (CRP), a standard inflammatory biomarker synthesised in the liver, is modulated by proinflammatory cytokines originating from adipocytes such as interleukin-6 (IL-6), and tumour necrosis factor α (TNF-α) (9). (58) reported that bacterial infections, trauma, surgery, and other inflammatory occurrences can cause CRP levels to rise up to 1000 times from baseline concentrations and then drop back to baseline within 12-14 days. Furthermore, the level of CRP can increase 100 to 200 times or more during acute systemic inflammation and remains persistently increased in individuals with T2DM. (59) indicated that people with T2DM exhibit CRP levels ranging from 4.49 to 16.48 mg/L. There is growing evidence that inflammation plays a critical role in T2DM pathogenesis and is associated with low-grade inflammation marked by high levels of inflammatory proteins such as CRP (11).

The current findings indicated that CRP levels exhibited a positive correlation with increasing age in both healthy individuals and patients with T2DM. The typical CRP values in patients with T2DM range from 4.73±0.71 to 11.32±0.79 mg/dL, depending on age. In healthy individuals, CRP levels range from 0.99±0.32 to 2.82±0.61 mg/dL. Additionally, both T2DM patients and healthy controls showed positive associations between CRP levels and advancing age groups, with correlation coefficients of $r = 0.9865$ and $r = 0.9916$, respectively. The findings are consistent with other prior research including (60),(61), and (62) which reveal a substantial positive correlation between increased CRP levels and the probability of developing T2DM.Increased serum CRP levels in male streptozotocin-induced Sprague-Dawley rats compared with untreated rats. (63) noted that elevated hs-CRP levels correlated with all markers of T2DM and insulin resistance.

(64) indicated that serum CRP is associated with the incidence of diabetic neuropathy, a common consequence of diabetes. Moreover, studies of (65) demonstrated that T2DM patients with DN had greater hs-CRP levels than both healthy people and T2DM patients without DN. Similarly, (66) discovered a correlation between elevated hs-CRP levels and diabetic neuropathy problems in patients with T2DM. (67) identified a positive association between increased hs-CRP levels in the blood and the development and worsening of diabetic retinopathy. (68) suggested that in individuals with T2DM peripheral neuropathy, elevated CRP levels have a positive correlation with the degree of inflammation. Consequently, serum hs-CRP levels may serve as a valuable indicator for assessing the risk of diabetic neuropathy in patients with T2DM (69).

Moreover, adipose tissue, particularly visceral white adipose tissue (WAT), significantly contributes to the inflammation that leads to and the progression of T2DM. Also, studies have shown that certain proinflammatory cytokines can make human adipocytes make CRP. This suggests that there may be a link between obesity and other health problem like insulin resistance (70). According to (10) a higher CRP level in diabetes individuals is also a favourable indicator of vascular problems and the advancement of cardiovascular disease. Furthermore, there is evidence linking CRP to microvascular problems like

retinopathy, nephropathy, and neuropathy brought on by diabetes. Increased glucose levels may induce microvascular changes and heightened synthesis of inflammatory mediators such as CRP, IL-6, and TNF-α (71). Several metabolic and inflammatory elements linked to the progression of T2DM such as elevated blood glucose, adipokines, and free fatty acid concentrations, may stimulate the synthesis of CRP. CRP is capable of detecting and binding to both endogenous damages associated molecular patterns (DAMPs) and exogenous pathogen-associated molecular patterns (PAMPs). Consequently, CRP triggers an immune response and aids in the removal of diverse infections and damaged necrotic or apoptotic cells (72). Fortunately, CRP also serves as a proinflammatory mediator, binding to Fc receptor of IgG and triggering the release of proinflammatory cytokines (73).

4.5 Homocysteine

Homocysteine, a sulfhydryl-containing amino acid, serve as an intermediary in the production of the amino acids methionine and cysteine (74). Hperhomocysteinemia is a clinical condition marked by an elevated concentration (> 15 μmol/L) of homocysteine in the bloodstream (75). The total content of fasting homocysteine in the plasma of healthy individuals ranges from 5.0 to 15.0 μmol/. Multiple studies have demonstrated modified serum homocysteine levels in patients with T2DM. (76) indicates that plasma concentrations of homocysteine are higher in patients with diabetes, especially those with T2DM and people with prediabetic conditions characterised by insulin resistance.

The current investigation revealed a substantial elevation in homocysteine levels associated with T2DM, although age does not show a significant impact. The mean homocysteine levels in people with T2DM vary from 9.45±0.97 to 20.49±0.92 μmol/dL with increasing age. In healthy individuals, homocysteine levels rise with age, often ranging from 7.53±0.85 to 17.82±0.84 μmol/dL. These outcomes are comparable to those of (77) who discovered that the diabetic group's average serum homocysteine concentration was 16.52±3.56 while the control group was 11.74±2.76 μmol/dL. There is a statistically significant correlation between these differences and a higher prevalence of macrovascular diseases in men as well as with advancing age. In a related study, (78.) found that the plasma homocysteine concentration was considerably higher in diabetic individuals with macroangiopathy, at 14.2±5.8 μmol/L. (79) discovered elevated homocysteine concentrations in individuals with T2DM and hypertension ($P<0.001$) compared to control individuals. (80) found that the amount of homocysteine in the blood was much higher in people with T2DM (15.1±4.7 μmol/L) than in the control group (8.9±2.96 μmol/L). They identified a strong and independent correlation between hyperhomocysteinemia and lipid profiles in T2DM, contributing to coronary heart disease. (81) also determined that serum homocysteine quantities can act as an independent risk factor for cardiovascular disorders in T2DM patients. (16) asserted that elevated serum homocysteine levels constitute a cardiovascular disease risk factor that is 1.6 times more pronounced in patients with T2DM compared to non-diabetic persons.

Multiple prior research studies, including those by(82), (83), and (84) identified a significant correlation between T2DM and hyperhomocysteinemia. (85) found a connection between retinopathy and neuropathy in patients with T2DM who also had microvascular disease and elevated homocysteine levels. (86) asserted that an increased serum content of homocysteine is anticipated to augment the synthesis of its oxidation derivatives, including Hcydisulfides and Hcythiolactone. These oxidative products cause excessive sulfation of collagen, which damages endothelial cells and exacerbates the development of arteriosclerosis and thrombosis. (87) state that hyperhomocysteinemia in T2DM may be a factor in the occurrence of hypertension and chronic vascular problems.(88) showed a substantial variance ($P<0.0001$) in blood homocysteine levels between diabetic patients with cardiovascular complications (27.20±6.02 μmol/L) compared to those without cardiovascular comorbidity (18.03±4.61 μmol/L).(89) found that diabetic individuals with hypertension had higher homocysteine concentrations than their normotensive counterparts. These results suggest that high hyperhomocysteinemia may have both atherogenic and thrombogenic effects, making people more likely to have adverse cardiovascular outcomes.

4.6 Adenosine deaminase

Adenosine deaminase (ADA) is an essential enzyme that is present in all human tissues and plays a role in the metabolism of purine nucleotides. It converts adenosine and deoxyadenosine into inosine and deoxyinosine respectively by catalysing their deamination. Since this enzyme is essential for the proliferation and differentiation of lymphocytes as well as the monocyte-macrophage system, it has been used as a biomarker for a number of immune system diseases (90).(91) suggested that adenosine concentrations are generally lower during normal conditions; however, they can exceed normal levels by greater than a hundred-fold when cells experience stress. Serum ADA controls adenosine, which promotes insulin activity via a number of mechanisms including lipid synthesis and glucose transfer. Consequently, elevated ADA levels lead to decreased adenosine levels, which in turn reduce glucose absorption in cells and heighten insulin resistance. (92) link the pathogenesis of T2DM and reduced ADA activity, which may enhance T-lymphocyte activity, insulin sensitivity, and inflammation.

The finding of the present investigation indicated a considerable increase in ADA levels concerning T2DM and age. The typical ADA levels in T2DM patients range from 33.26±3.81 to 46.64±2.54 U/L, depending on age. In healthy individuals, ADA levels range from 8.37±0.99 to 20.09±1.38 U/L. Additionally, both T2DM patients and healthy controls showed positive correlations between ADA levels and increasing age groups, with correlation coefficients of $r = 0.9311$ and $r = 0.9452$, respectively. These findings are consistent with those of (93) who found that patients with diabetic feet had significantly higher serum concentrations of ADA, HbA1c, and neutrophil percentages than individuals without the condition. The averages of these levels were reported as 71.79±25.11 IU/L, 11.53±3.11% and 75.91±9.77% respectively. Similarly, (98) showed increased ADA activity in the serum of patients with T2DM. (94) investigated the activity of adenosine deaminase in T2DM and found that elevated blood ADA levels correlated with increased HbA1c levels, potentially influencing the glycaemic state of diabetes. (95) indicated that markedly elevated levels of ADA in patients relative to controls imply that ADA is implicated in the pathogenesis of T2DM and its consequences. Contrary to the current findings, (96) showed that the activity of 5'-nucleotidase and ADA remained unchanged in isolated glomeruli of streptozocin-induced diabetic rats.

(97) stated that chronic hyperglycemia raises ADA levels and causes oxidative stress and insulin resistance by causing the Nicotinamide Adenine Dinucleotide Phosphate Hydrogen (NADPH) oxidase system to produce enediol radical and superoxide ions. Consequently, an increase in ADA activity leads to a decrease in adenosine concentration, which in turn reduces the absorption of glucose into cells (98). Adenosine improved insulin and contraction-induced glucose transport in skeletal muscle by making it easier for GLUT-4 to rise at the cell membrane. There is correlation between enhanced sensitivity to insulin and adenosine A1 receptor agonists. Consequently, the reduction of reduction of adenosine resulting from heightened adenosine deaminase activity could result in elevated insulin resistance and eventual hyperglycemia, an important characteristic of T2DM (99). Furthermore, numerous investigations have determined that adenosine has effects analogous to insulin on glucose and lipid metabolism within adipose tissue (100). (101) found that decreased adenosine levels in insulin-sensitive tissue caused by enhanced ADA activity led to decreased cell uptake of glucose.

5. Declarations

Ethics Approval and Consent to Participate

The study was conducted in accordance with the ethical standards of the institutional research committee and informed written consent was obtained from all participants prior to sample collection.

Competing Interests

The authors declare that they have no competing interests.

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