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Original Article

Analysis and correlation of Blood HbA1c percentages with microalbuminuria and dyslipidemia among diabetes in a tertiary care multi-speciality hospital of Eastern India.

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ABSTRACT

Background:
Diabetes is a metabolic chronic disorder that is characterised by high glucose in the blood. This may lead to various problems in the body that includes renal failure. The HbA1c test is used to diagnose and monitor diabetes, as it is a measure of long-term glucose control. The presence of microalbuminuria in urine is used as an early sign of diabetic nephropathy. Elevated HbA1c levels are associated with an increased risk of microalbuminuria and diabetic nephropathy. The relationship between HbA1c and microalbumin in urine is complex and not fully understood, but hyperglycemia-induced oxidative stress, endothelial dysfunction, and inflammation are proposed as potential mechanisms.

Methodology:
Our study aimed to investigate the relationship between HbA1c levels and microalbumin in urine, serum cholesterol, and serum tri-glycerides in different age groups. Two groups of patients were formed based on their HbA1c values, and each group was further divided into three age groups. The blood and urine samples were already being collected and analyzed, and the data were retrospectively analyzed.

Results:
The results showed no significant differences in urine microalbumin levels with blood HbA1c levels ≥ 6.5%. However, significant differences were observed among the means of urine microalbumin and serum cholesterol levels with blood HbA1c levels < 6.5%. There were also noticeable differences in serum cholesterol and serum tri-glyceride levels in different age groups with blood HbA1c levels ≥ 6.5%.

Conclusion:
Overall, the study suggests that there is an association between serum cholesterol and serum tri-glyceride levels and high levels of blood HbA1c% in different age groups. However, there was no significant association observed between urine microalbumin levels and high HbA1c levels. This study suggests that early detection of microalbuminuria and intervention to reduce serum cholesterol levels may be important in reducing the risk of diabetic nephropathy and cardiovascular disease (CVD) in individuals with Type II diabetes mellitus (T2DM).

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INTRODUCTION

Diabetes is a metabolic chronic disorder that is characterised by high glucose in the blood, this may lead to various problems in the body that includes renal failure. It includes two types which includes type 1 which is insulin dependent and involves lifelong problem of insulin production in the body. The other type being the type II diabetes is the most common one and is insulin independent. It is more dependent on dietary habits of individuals. WHO estimates that by 2030 diabetes will be the 7th leading cause of death. (Global status report)

The type II diabetes mellitus effects millions of people worldwide.

If the diabetes is not controlled adequately then it may lead to various serious complications like diabetic nephropathy, a condition with kidney damage and proteinuria. As a confirmatory test HbA1c test done through HPLC method where glycated hemoglobin or HbA1c is used to diagnose and monitor diabetes because it a measure of long term glucose control [1]. Glycated haemoglobin A1c is a component of red blood cells. It goes through a non-enzymatic reaction where alpha-amino groups of the valine residues at the N-terminus of the beta-chains of human haemoglobin interact with the glucose molecule. The presence of small amount of albumin in urine termed as microalbuminuria, is used as an early sign of diabetic nephropathy with an increased risk of cardiovascular disease and even mortality [2].

Studies have given evidences that elevated HbA1c levels are associated with an increased risk of microalbuminuria with progression to diabetic nephropathy [3]. The main functional relationship between HbA1c and microalbuminuria is quite complex and the mechanism underlying is not yet fully understood. Some studies suggest that hyperglycemia-induced oxidative stress may play a role in the development of microalbuminuria [4], while others propose that endothelial dysfunction and inflammation may also contribute to this process [5].

Seeing the importance of microalbuminuria as a predictive measure of diabetic nephropathy and cardiovascular disease, the relationship or correlation of HbA1c with microalbumin in urine is crucial.

Method –

Patient subjects:

Our total study involved patients over the course of a year from March 2022 to March 2023.Two groups were made based on the glycated HbA1c values. One group was with HbA1c values that didn't exceeded 6.4. The other group was of patients with HbA1c values 6.5 and higher. Each of the groups were further sub divided into 3 groups that were based on ages. The groups hence formed were of 20-40, 41-60 and 61-75 age groups.

Inclusion criteria: -

Male/Female, HbA1c <6.5 and >6.5, patients of ages from 20 to 75, patients with both lipid profiles and urinary microalbumin test data.

Exclusion criteria: -

Patients below age 20 and above 75, patients without any of the required tests or only having either lipid profile or urinary microalbumin data.

Our whole data collection and further inference was totally based on retrospective analysis and hence required no such approval from the ethical committee.

Sample sorting

Prior to our investigation the blood samples as well as urine samples were already being collected and analysed on their respective machines Bio-rad and Cobas 6000. In CMRI the HbA1c molecule detected and calculated in blood through HPLC method by Bio-rad D-10. HbA1c is directly measured with ion exchange high performance liquid chromatography on Bio-rad D-10 which detect the molecule without interference of HbS, HbC, HbD, HbF and HbE heterozygotes. The microalbumin was detected in the Cobas 6000 machine using appropriate reagents. Along with microalbumin the serum cholesterol and serum tri-glycerides were also checked for any relation with HbA1c. The latter two, also being measured by Cobas 6000 with their respective reagents.

So the already measured parameters were available to us in the databases and we took data from their as per our needs. Only those patients with all the required tests were used for our analysis.

Results

A total of 267 patients' data were found to be relevant with our analysis and were hence taken into consideration. Out of these 119 were those with HbA1c levels less than 6.5 and 148 were those with HbA1c levels greater than 6.5.

The groups were further divided into three age groups for better understanding of the changes that may occur as a result of changing age.

One-way ANOVA analysis of Blood HBA1C% ≥ 6.5%, in different age groups showed no significant difference among means [p=0.294]

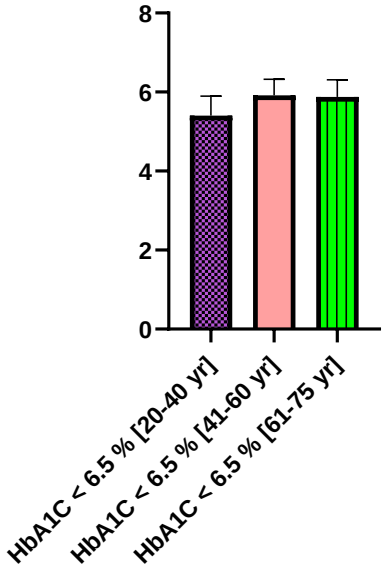
Table 1: Descriptive Statistics of Blood HbA1C% [< 6.5%] in different age groups

Statistical parameters	HbA1C < 6.5 % [20-40 yrs.] n=23	HbA1C < 6.5 % [41-60 yrs.] n=50	HbA1C< 6.5 % [61-75 yrs.] n=46
Minimum	4.300	4.800	4.800
25% Percentile	5.100	5.700	5.575
Median	5.400	6.000	5.950
75% Percentile	5.800	6.300	6.300
Maximum	6.200	6.400	6.400
Range	1.900	1.600	1.600
Mean	5.400	5.916	5.876
Std. Deviation	0.4936	0.4093	0.4356
Std. Error of Mean	0.1029	0.05788	0.06423

One-way ANOVA analysis of Blood HBA1C% ≥ 6.5%, in different age groups showed no significant difference among means [p=0.294]

HbA1C % [< 6.5%] in different age groups

1(a)



HbA1C % [> 6.5%] in different age groups

1(b)

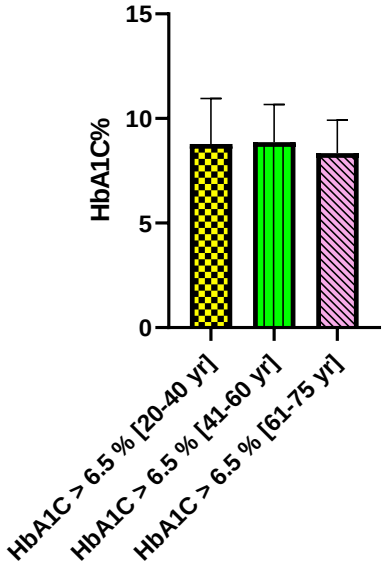


Fig: 1 (a) & (b) bar diagram of the HbA1c distribution (in %) in different age groups with HbA1c <6.5 and >6.5 respectively.

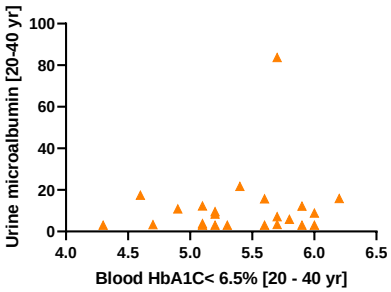
One-way ANOVA analysis of Blood HBA1C% < 6.5%, in different age groups showed significant difference among means [p<0.0001].

Table 2: Descriptive Statistics of Blood HbA1C% [≥ 6.5%] in different age groups

Statistical parameters	**HbA1C ≥ 6.5 % [20-40 yrs.] n=33**	**HbA1C ≥ 6.5 % [41-60 yrs.] n=63**	**HbA1C ≥ 6.5 % [61-75 yrs.] n=52**
Minimum	6.500	6.500	6.500
25% Percentile	7.150	7.300	7.125
Median	8.200	8.400	7.900
75% Percentile	9.550	10.20	9.275
Maximum	16.80	13.50	14.00
Range	10.30	7.000	7.500
Mean	8.776	8.867	8.350
Std. Deviation	2.174	1.802	1.567
Std. Error of Mean	0.3785	0.2270	0.2174

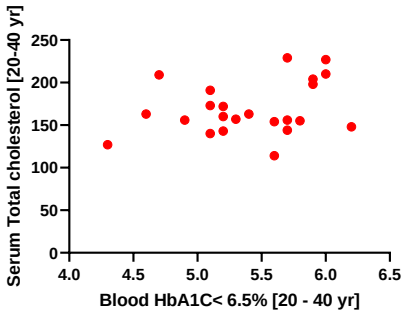
One-way ANOVA analysis of Blood HBA1C% < 6.5%, in different age groups showed significant difference among means [p<0.0001].

Correlation of Blood HbA1C % with urine micro albumin [mg/L]



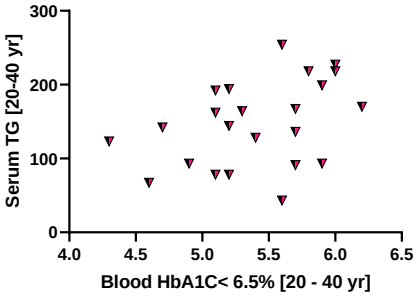
2(a)

Correlation of Blood HbA1C % with serum total cholesterol [mg/dL]



2(b)

Correlation of Blood HbA1C % with serum triglyceride [mg/dL]



2(c)

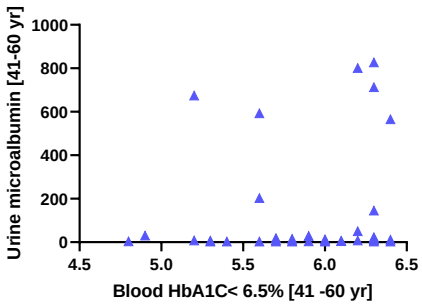
In all these statistical analyses a p value of ≤0.05 was considered to be statistically significant.
The statistical analysis was done of the data hence provided and Kruskal-Wallis analysis was done of the data.
Kruskal-Wallis analysis of Urine microalbumin with Blood HBA1C% < 6.5%, in different age groups showed significant difference among means [p=0.011].

Table 3: Descriptive Statistics of Urine microalbumin with Blood HbA1C% [< 6.5%] in different age groups

Statistical parameters	Urine microalbumin [mg/L] [20-40 yrs.] n=23	Urine microalbumin [mg/L] [41-60 yrs.] n=50	Urine microalbumin [mg/L] [61-75 yrs.] n=46
Minimum	3.000	3.000	3.000
25% Percentile	3.000	3.083	3.988
Median	7.110	7.400	21.77
75% Percentile	12.31	19.93	252.5
Maximum	83.71	827.2	741.0
Range	80.71	824.2	738.0
Mean	11.36	97.95	168.6
Std. Deviation	16.74	228.1	240.7
Std. Error of Mean	3.490	32.26	35.49

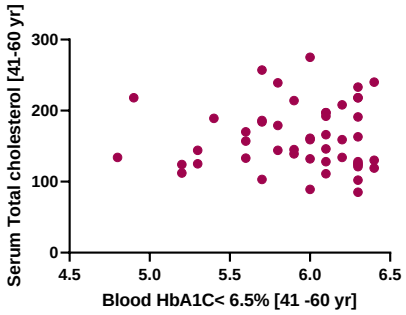
Kruskal-Wallis analysis of Urine microalbumin with Blood HBA1C% < 6.5%, in different age groups showed significant difference among means [p=0.011].

Correlation of Blood HbA1C % with urine micro albumin [mg/L]



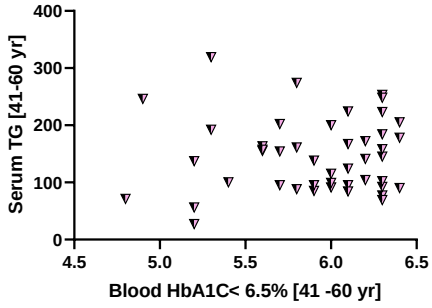
3(a)

Correlation of Blood HbA1C % with serum total cholesterol [mg/dL]



3(b)

Correlation of Blood HbA1C % with serum triglyceride [mg/dL]



3(c)

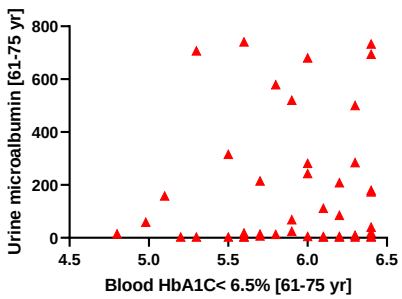
Kruskal-Wallis analysis of Serum cholesterol with Blood HBA1C% < 6.5%, in different age groups showed significant difference among means [p=0.0052].

Table 4: Descriptive Statistics of Serum cholesterol with Blood HbA1C% [< 6.5%] in different age groups

Statistical parameters	Serum cholesterol [mg/dL] [20-40 yrs.] n=23	Serum cholesterol [mg/dL] [41-60 yrs.] n=50	Serum cholesterol [mg/dL] [61-75 yrs.] n=46
Minimum	114.0	85.00	67.00
25% Percentile	148.0	125.0	112.0
Median	160.0	151.5	133.0
75% Percentile	198.0	193.3	163.5
Maximum	229.0	275.0	228.0
Range	115.0	190.0	161.0
Mean	169.3	161.2	139.7
Std. Deviation	31.08	45.83	39.29
Std. Error of Mean	6.480	6.481	5.793

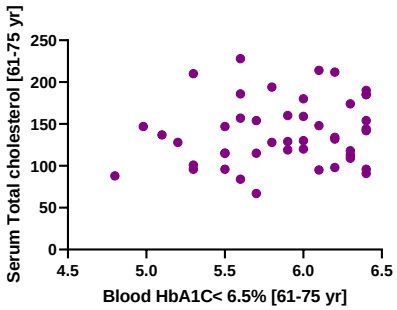
Kruskal-Wallis analysis of Serum cholesterol with Blood HBA1C% < 6.5%, in different age groups showed significant difference among means [p=0.0052].

Correlation of Blood HbA1C % with urine micro albumin [mg/L]



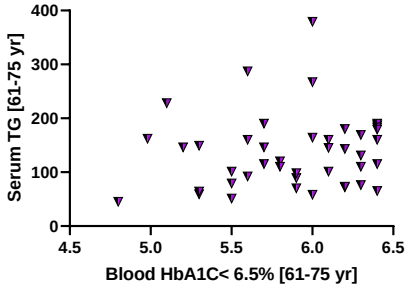
4(a)

Correlation of Blood HbA1C % with serum total cholesterol [mg/dL]



4(b)

Correlation of Blood HbA1C % with serum triglyceride [mg/dL]



4(c)

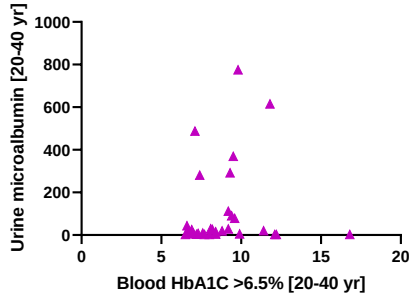
Kruskal-Wallis analysis of Serum TG with Blood HBA1C% < 6.5%, in different age groups showed no significant difference among means [p=0.482].

Table 5: Descriptive Statistics of Serum TG with Blood HbA1C% [< 6.5%] in different age groups

Statistical parameters	Serum TG [mg/dL] [20-40 yrs.] n=23	Serum TG [mg/dL] [41-60 yrs.] n=50	Serum TG [mg/dL] [61-75 yrs.] n=46
Minimum	43.00	27.00	45.00
25% Percentile	93.00	94.25	79.00
Median	144.0	139.5	125.5
75% Percentile	194.0	194.0	171.5
Maximum	254.0	319.0	379.0
Range	211.0	292.0	334.0
Mean	147.0	145.8	135.3
Std. Deviation	57.65	64.69	67.00
Std. Error of Mean	12.02	9.149	9.879

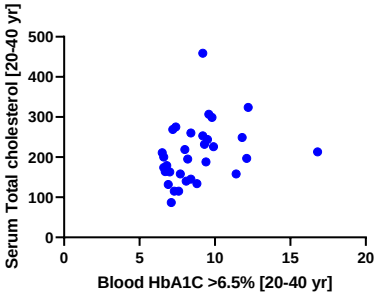
Kruskal-Wallis analysis of Serum TG with Blood HBA1C% < 6.5%, in different age groups showed no significant difference among means [p=0.482].

Correlation of Blood HbA1C % with urine micro albumin [mg/L]



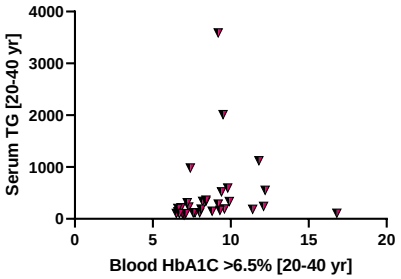
5(a)

Correlation of Blood HbA1C % with serum total cholesterol [mg/dL]



5(b)

Correlation of Blood HbA1C % with serum triglyceride [mg/dL]



5(c)

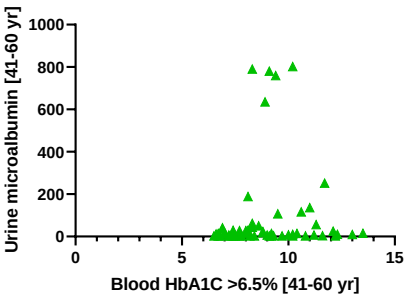
Kruskal-Wallis analysis of Urine microalbumin with Blood HBA1C% ≥ 6.5%, in different age groups showed no significant difference among means [p=0.317].

Table 6: Descriptive Statistics of Urine microalbumin with Blood HbA1C% [≥ 6.5%] in different age groups

Statistical parameters	Urine microalbumin [mg/L] [20-40 yrs.] n=33	Urine microalbumin [mg/L] [41-60 yrs.] n=63	Urine microalbumin [mg/L] [61-75 yrs.] n=52
Minimum	3.000	3.000	3.000
25% Percentile	4.715	3.000	4.120
Median	19.46	11.59	32.93
75% Percentile	84.60	37.03	109.6
Maximum	775.4	802.4	726.9
Range	772.4	799.4	723.9
Mean	103.7	84.57	119.3
Std. Deviation	193.6	203.7	200.4
Std. Error of Mean	33.69	25.66	27.79

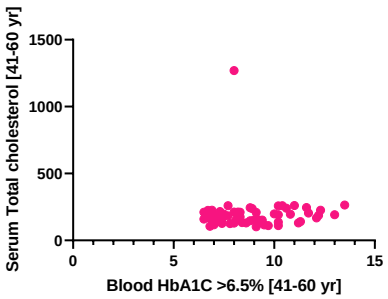
Kruskal-Wallis analysis of Urine microalbumin with Blood HBA1C% ≥ 6.5%, in different age groups showed no significant difference among means [p=0.317].

Correlation of Blood HbA1C % with urine micro albumin [mg/L]



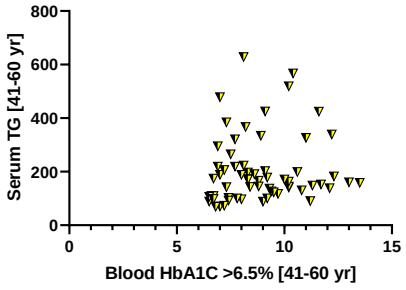
6(a)

Correlation of Blood HbA1C % with serum total cholesterol [mg/dL]



6(b)

Correlation of Blood HbA1C % with serum triglyceride [mg/dL]



6(c)

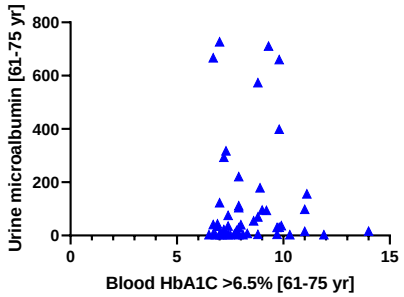
Kruskal-Wallis analysis of Serum cholesterol with Blood HBA1C% ≥ 6.5%, in different age groups showed significant difference among means [p<0.0001].

Table 7: Descriptive Statistics of Serum Cholesterol with Blood HbA1C% [≥ 6.5%] in different age groups

Statistical parameters	Serum cholesterol [mg/dL] [20-40 yrs.] n=33	Serum cholesterol [mg/dL] [41-60 yrs.] n=63	Serum cholesterol [mg/dL] [61-75 yrs.] n=52
Minimum	87.00	102.0	76.00
25% Percentile	151.5	140.0	111.8
Median	197.0	183.0	139.0
75% Percentile	251.0	212.0	176.8
Maximum	459.0	1269	295.0
Range	372.0	1167	219.0
Mean	206.8	196.6	148.0
Std. Deviation	74.91	144.4	52.31
Std. Error of Mean	13.04	18.19	7.254

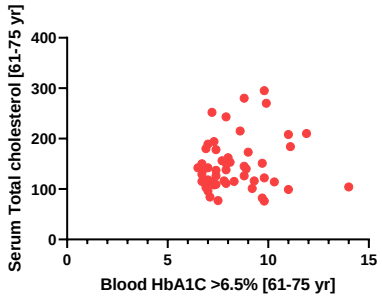
Kruskal-Wallis analysis of Serum cholesterol with Blood HBA1C% ≥ 6.5%, in different age groups showed significant difference among means [p<0.0001].

Correlation of Blood HbA1C % with urine micro albumin [mg/L]



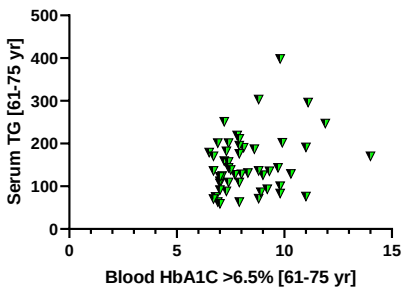
7(a)

Correlation of Blood HbA1C % with serum total cholesterol [mg/dL]



7(b)

Correlation of Blood HbA1C % with serum triglyceride [mg/dL]



7(c)

Fig: 5,6,7- Correlation of blood HbA1C% with Urine Microalbumin, Serum total Cholesterol and Serum Triglyceride levels done by applying Spearman correlation test and scatter plot of the analysis is shown in the figure. Here the HbA1c level is >6.5 and the test is repeated for each age group (a,b,c as shown)

Kruskal-Wallis analysis of Serum TG with Blood HBA1C% ≥ 6.5%, in different age groups showed significant difference among means [p=0.0027].

Table 8: Descriptive Statistics of Serum TG with Blood HbA1C% [≥ 6.5%] in different age groups

Statistical parameters	Serum TG [mg/dL] [20-40 yrs.] n=33	Serum TG [mg/dL] [41-60 yrs.] n=63	Serum TG [mg/dL] [61-75 yrs.] n=52
Minimum	73.00	69.00	59.00
25% Percentile	118.5	109.0	101.8
Median	200.0	163.0	136.0
75% Percentile	350.0	219.0	189.3
Maximum	3589	628.0	398.0
Range	3516	559.0	339.0
Mean	435.4	201.2	150.0
Std. Deviation	684.1	126.4	67.54
Std. Error of Mean	119.1	15.92	9.366

Kruskal-Wallis analysis of Serum TG with Blood HBA1C% ≥ 6.5%, in different age groups showed significant difference among means [p=0.0027].

Discussion

Our study used Kruskal-Wallis analysis to determine if there is any statistical significance of differences among the means of the various groups. The results showed that there were noticeable differences among the means of serum cholesterol and serum TG levels in different age groups with blood HBA1C% levels ≥ 6.5%. On the other hand, no significant differences were found among the means of urine microalbumin levels with blood HBA1C% levels ≥ 6.5%. In contrast, significant differences were observed among the means of urine microalbumin and serum cholesterol levels with blood HBA1C% levels < 6.5%. In contrast, no significant differences were observed among the means of serum TG levels with blood HBA1C% levels < 6.5%.

The results suggest that there are some association of serum cholesterol and serum TG with high levels of blood HbA1c% in different age groups, while no such association was observed for urine microalbumin levels. These findings are consistent with previous studies that have reported the association between serum lipids and high blood glucose levels in individuals with type 2 diabetes (T2D) [6]. The findings also suggest that monitoring serum cholesterol and serum TG levels could be important in managing T2D and preventing its complications, such as cardiovascular diseases [7].

The lack of a significant association between urine microalbumin levels and high blood HBA1C% levels in the present study contrasts with some previous studies [8]. This suggests that further studies are needed to determine the relationship between urine microalbumin levels and high blood glucose levels.

Based on the Kruskal-Wallis analysis of urine microalbumin, serum cholesterol, and serum triglyceride levels in individuals with blood HbA1c levels below 6.5% in different age groups, significant differences were observed in urine microalbumin and serum cholesterol levels, while no significant difference was observed in serum triglyceride levels.

Microalbuminuria is a marker of early renal damage and is associated with an increased risk of cardiovascular disease in individuals with type 2 diabetes mellitus (T2DM) [9]. Elevated microalbumin levels in the urine are an indication of kidney damage and are associated with an increased risk of cardiovascular disease and diabetic nephropathy [10]. The Kruskal-Wallis analysis revealed that there was a significant difference among the means of urine microalbumin levels in individuals with blood HbA1c levels below 6.5% in different age groups. This suggests that early detection of microalbuminuria may be important in identifying individuals at risk of developing diabetic nephropathy and cardiovascular disease.

Cholesterol is a major risk factor for cardiovascular disease (CVD) in individuals with T2DM [11]. Dyslipidemia, including high levels of low-density lipoprotein cholesterol (LDL-C) and triglycerides, and low levels of high-density lipoprotein cholesterol (HDL-C), is commonly observed in individuals with T2DM [12]. The Kruskal-Wallis analysis revealed that there was a significant difference among the means of serum cholesterol levels in individuals with blood HbA1c levels below 6.5% in different age groups. This suggests that early intervention to reduce serum cholesterol levels may be important in reducing the risk of CVD in individuals with T2DM.

In contrast, the Kruskal-Wallis analysis revealed that there was no significant difference among the means of serum triglyceride levels in individuals with blood HbA1c levels below 6.5% in different age groups. This suggests that serum triglyceride levels may not be a reliable predictor of CVD risk in individuals with T2DM, particularly in the early stages of the disease.

Conclusion

The present study suggests that serum cholesterol and serum TG levels are associated with high blood HBA1C% levels in different age groups, while no significant association was found between urine microalbumin levels and high blood HBA1c% levels. These findings have implications for the management of Type II Diabetes and its complications, particularly cardiovascular diseases. This study suggests that early detection of microalbuminuria and intervention to reduce serum cholesterol levels may be important in reducing the risk of diabetic nephropathy and cardiovascular disease (CVD) in individuals with Type II diabetes mellitus (T2DM). Further studies are needed to confirm these findings and to identify other risk factors that may be associated with an increased risk of diabetic nephropathy and CVD in individuals with T2DM.

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Conflict of Interest: Nil

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