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GLYCATED ALBUMIN AND HBA1C COMBINE ESTIMATION HELP IN BETTER DETECTION OF DYSGLYCEMIA IN HEALTHY ADULTS

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ABSTRACT

INTRODUCTION: Obesity is a pathophysiological condition resulting in many metabolic disorders affecting carbohydrate, protein and lipid metabolism. Increased waist circumference is associated with higher BMI. Glycated serum proteins (GSP) such as Glycated Albumin (GA) and HbA1c are formed by a non-enzymatic reaction where blood glucose binds with plasma proteins. GA allows earlier detection of rapid changes in blood glucose over 2-3 weeks.

METHODS: The study included 80 subjects of age between 20-40 years, 40 of them were obese (group I) and 40 were apparently healthy subjects (group II). Blood samples were collected for Lipid profile & GA in a red-capped vacutainer for and in purple capped vacutainer for HbA1c. HbA1c and Lipid Profile were measured using standard autoanalyzer-based kit methods. GA was measured using the ELISA technique.

RESULTS: Compared with groupI, groupII had significantly lower mean waist circumference($P<0.01$), triglycerides, total cholesterol($P<0.05$), LDL cholesterol($P<0.05$) and fasting blood glucose($P<0.05$). The Mean value of HbA1c was 5.53±0.64% and 5.02±0.48% ($P<0.001$) and GA was 14.84±5.43% and 15.89±6.10% in group I and II respectively. GA was positively correlated with HDL cholesterol($r=0.12$) in groupI and with total cholesterol($r=0.14$) and LDL($r=0.13$) cholesterol in groupII.

DISCUSSION AND CONCLUSION: Central obesity can identify the risk of metabolic and cardiovascular diseases. The glycation rate of albumin is higher than hemoglobin, thus GA detects earlier rapid changes in blood glucose. Our study findings concluded that subjects with BMI≥30 had lower GA levels and high HbA1c levels. Hence, the estimation of GA in non-obese individuals serves as an early accurate marker to detect dysglycemia.

KEYWORDS: Obesity, Body Mass Index (BMI), Glycated Hemoglobin (HbA1c), Glycated Albumin, Lipid Profile, Dysglycemia

INTRODUCTION

Obesity is a pathophysiologic state in which energy intake and energy expenditure are out of balance, ultimately leading to an increased risk for several diseases. With the rapid development of social, economic and corresponding changes in diet and physical activity, the increasing prevalence of obesity is becoming a worldwide health concern [1]. Measuring fat and estimating its level in the body, is very difficult. So, when studying a large number of populations, body height and weight are mostly used for fat estimation [2]. The most common is Quetelet's index, also known as body mass index (BMI), which is body weight (kg) divided by height squared (m2) [3].

Rapid changes in lifestyle, food habits and living conditions of societies generated various changes which led to the generation of many chronic diseases like hypertension, obesity, type 2 diabetes, dyslipidemias, and metabolic syndrome, which are prevalent in all age groups [4,5].

Glycated hemoglobin (hemoglobin A1c, HbA1c, A1c, or Hb1c; also known as HbA1c or HGBA1c) is a form of hemoglobin that is measured primarily to identify the average plasma glucose concentration over prolonged periods [6]. HbA1c is formed by irreversible glycation of hemoglobin at N-terminals of beta chain valine. HbA1c is considered a marker of diabetic control except in the following situations. HbA1c is a reliable indicator of diabetic control except in the following situations: [7] (a) Certain Hemoglobinopathies and red cell disorders, myelodysplastic disease. (b) Interference with the test (this depends on the method used: persistent fetal hemoglobin and hemoglobin variants, carbamylated hemoglobin (uremic patients). (c) In patients who fluctuate between very high and very low levels of glycated hemoglobin in that case readings can be misleading (the clinician should compare with extra information obtained from home capillary blood glucose tests). (d) HbA1c can be useful in identifying patients who may be presenting an unrealistically good report of their home glucose tests [7].

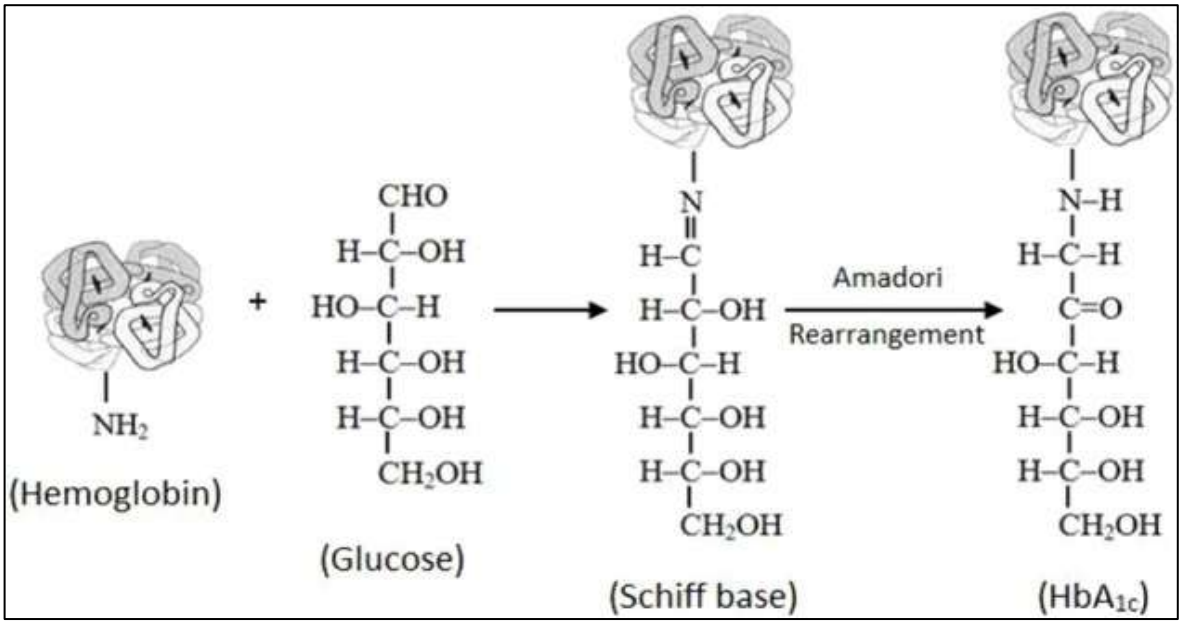

Figure 1. Formation of HbA1c [8]

Fructosamine (FA) has been studied as an alternate glycemic marker for diabetes screening and may be potentially useful for diagnosing prediabetes [9]. Since it reflects average blood glucose concentrations over the previous 1–4 weeks, it can be a useful clinical marker of short-term glycemic fluctuation and glucose control [10]. FA is especially useful in conditions that affect hemoglobin reliability and has moderate sensitivity and high specificity. FA could be taken as an indicator of the risk of developing microvascular complications. Thus, in conditions where HbA1c is not reliable, FA could be a valuable complementary marker [11].

The set of ketamine formed by non-enzymatic glycation of proteins is chemically called "Fructosamine". Glycated Albumin (GA) contributes about 80% of the total glycated protein among plasma Fructosamine [12]. Serum glycated albumin levels reflect the average serum glucose levels in two to three weeks prior to the measurement & are thought to be a powerful indicator of glycemic control over the span of a month. The glycation depends on the degree and duration of hyperglycemia [13]. Extracellular proteins, such as albumin, may be more susceptible to Amadori rearrangements than intracellular proteins such as Hemoglobin. The possible reason this could be that the plasma proteins are exposed to plasma glucose. These features could justify the differences in the rates of albumin glycation, which are about 9 to 10 times greater than those of hemoglobin [14].

MATERIAL AND METHODS

Study population

The present study was conducted in the Department of Biochemistry in collaboration with the Department of Medicine, Pt. B. D. Sharma PGIMS, Rohtak. For the study, 80 subjects out of which 40 were obese (BMI≥30) and 40 were nonobese (BMI<25) individuals in the age group of 20-40 years attending the Medicine outpatient department were enrolled.

Exclusion criteria: Subjects suffering from chronic diseases of organs like kidney, liver, malignancies, endocrinal disorders like diabetes were excluded from the study. Subjects taking any drugs, supplements, systemic steroids and sex hormones were not taken into account. Patient with any weight reduction surgery or with any psychiatric illness were also excluded

Statement of Ethics

The institutional ethical committee approved the protocol for this study. Informed written consent was provided by all the participants prior to study participation.

METHODOLOGY

Anthropometric measurements were taken in all study subjects. All participants underwent complete physical examinations including measurement of weight, height, waist, and hip circumferences. The weight of the individuals was measured using a standard and calibrated weighing scale with at least difference of 5 grams. Subjects were asked to stand upright on the weighing scale without shoes and with minimal clothing. Height was measured using a standard and calibrated stadiometer. The subject was asked to stand without shoes, with his head, shoulder blades, buttocks and heel touching the stadiometer so that they are in the same plane. Body mass index was calculated as weight(kg) divided by height2 in meters. The waist circumference was measured on the mid-axillary line between the inferior margin of the 12th rib and the iliac crest. Hip circumference was measured around the widest portion of the buttocks with the tape parallel to the floor.

Sample collection

After overnight fasting blood samples were collected aseptically by venipuncture from the antecubital vein in a plain red-capped vacutainer (4mL) and ethylene diamine tetra acetic acid (EDTA) vacutainer (2mL). Blood samples were processed within one hour of collection. Samples were subjected to centrifugation at 2000 rpm for 10 minutes and serum was separated, Triglycerides, total cholesterol, high-density lipoprotein-cholesterol (HDL-c) and Glycated Hemoglobin (HbA1c) were analyzed on Randox autoanalyzer using a standard kit-based method (Randox Laboratories Limited, Crumlin Country Antrim, United Kingdom) [15-17]. Very low-density lipoprotein – cholesterol (VLDL-c) and low-density lipoprotein cholesterol (LDL-c) were calculated from the Friedewald formula as follows: VLDL-c (mg/dL) = TG (mg/dL)/5 and LDL-c (mg/dL) = TC (mg/dL) − HDL-c (mg/dL) − TG (mg/dL)/5. Glycated hemoglobin estimation was based on the principle of turbidimetric inhibition immunoassay (TINIA) for hemolysed whole blood. Glycated Albumin was measured by Enzyme-Linked Immunosorbent Assay (ELISA) technique using a biotin-labeled antibody and streptavidin protein that is covalently conjugated to horseradish peroxidase (HRP) reagents (Wuhan Fine Biotech Co Ltd, Wuhan, China) [18].

Statistical analysis

All statistical analysis was performed by using Microsoft Excel 2019 and other appropriate online available software (JASP 0.19.2). Results were expressed as the mean and standard deviation of the mean. Statistical tests including unpaired student's t-test; two-tailed. Pearson correlation tests (r) between the study variables were calculated. Data were considered to be significant if $p<0.05$ and highly significant with $p<0.01$.

RESULTS

Data from 80 subjects were used in this cross-sectional analysis. The mean age in group I (obese) was 32.55 ± 6.43 years and in group II (non-obese) was 29.33 ± 5.88 years and was comparable between both groups ($p >0.05$).

Anthropometric measurements, clinical and biochemical characteristics of the study subjects are displayed in Table 1 and Table 2. When compared with group I, group II had significantly lower mean waist circumference, hip circumference (both $P<0.01$), triglycerides, total cholesterol ($P<0.05$) and LDL cholesterol ($P<0.05$) but HDL cholesterol showed a higher value in group II. Mean fasting blood glucose was 93.6 ± 12.97 mg/dL and 87.35 ± 12.27 mg/dL in group I and group II respectively ($P<0.05$). The mean value of glycated albumin was higher in group II when compared with group I as 15.89 ± 6.10 % and 14.84 ± 5.43 % respectively. In obese individuals, the mean value of HbA1c was higher i.e. 5.53 ± 0.64 % when compared with non-obese 5.02 ± 0.48 % ($p<0.001$). The mean value of serum albumin did not show any significant difference in both groups. BMI was positively correlated with glycated albumin in group II whereas in group I it was negatively correlated with glycated albumin (Figures 2 & 3). HbA1c did not show any significant correlation with lipid profile in group I while in group II HbA1c was positively correlated with Triglycerides (r=0.248), total cholesterol (r=0.404) and LDL-cholesterol (r=0.468) (Table 3). In group I there is no significant correlation observed between glycated albumin and lipid profile while a significant positive correlation was observed between glycated albumin with total cholesterol (r=0.14) and LDL cholesterol (r=0.29) in group II (Figure 4). The mean age observed in obese was 32.55 and in non-obese was 29.33 (Figure 7).

Table 1: Anthropometric measurements in Group I and group II

Variables	Group I (Obese) Mean ± SD	Group II (Non-obese) Mean ± SD	T-test	p-Value
Age (years)	32.55 ± 6.43	29.33 ± 5.88	2.34	-
BMI	33.14 ± 3.66	21.09 ± 2.41	17.39	<0.0001***
Waist circumference (CM)	98.90 ± 8.31	77.91 ± 8.24	11.34	<0.0001***
Hip circumference (CM)	109.03 ± 13.86	84.42 ± 8.25	9.65	<0.0001***
WHR (Waist to Hip Ratio)	0.92 ± 0.18	0.93 ± 0.05	-0.34	>0.05**
*** – Statistically highly significant ** - Statistically non-significant # Independent t-test				

Table 2: Clinical and biochemical characteristics of the study subjects

Variables	Group I (Obese) Mean ± SD	Group II (Non-obese) Mean ± SD	T-test#	p-Value

Fasting Blood Glucose(mg/dL)	93.6 ± 12.97	87.35 ± 12.27	2.21	<0.05*
Albumin (g/dL)	4.45 ± 0.59	4.44 ± 0.60	0.08	>0.05**
Glycated Albumin (%) (GA)	14.84 ± 5.43	15.89 ± 6.10	-0.81	>0.05**
Glycated Hemoglobin (%) (HbA1c))	5.53 ± 0.64	5.02 ± 0.48	4.04	<0.0001***
Triglyceride (mg/dL)	167.15 ± 75.70	152 ± 89.94	0.81	>0.05**
Total Cholesterol (mg/dL)	184.65 ± 42.43	162.98 ± 48.83	2.12	<0.05*
HDL Cholesterol (mg/dL)	39.97 ± 11.04	40.3 ± 14.04	-0.12	>0.05**
LDL Cholesterol (mg/dL)	114.25 ± 37.94	91.9 ± 38.78	2.61	<0.05*
VLDL Cholesterol (mg/dL)	32.03 ± 15.64	30.78 ± 18.33	0.33	>0.05**

* – Statistically significant
*** – Statistically highly significant
** – Statistically non-significant
# Independent t-test

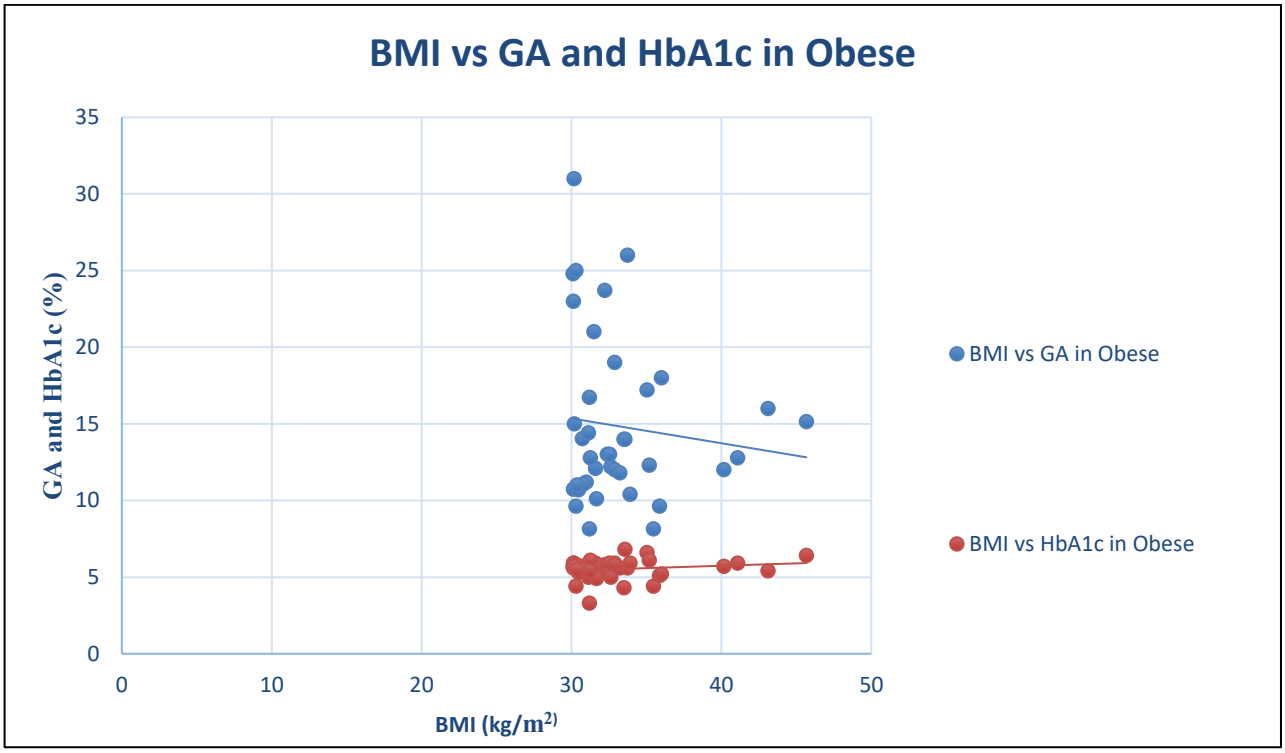

Figure 2: Graph showing the correlation of BMI with GA and HbA1c in Group I (Obese)

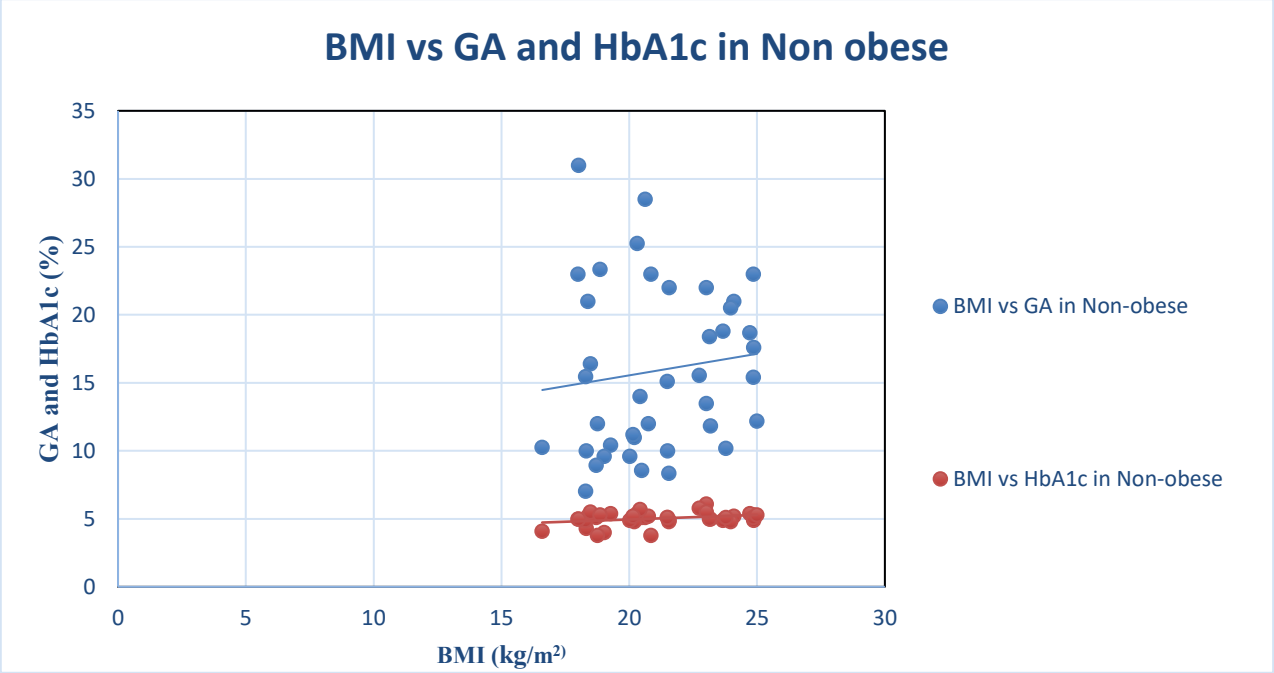

Figure 3: Graph showing the correlation of BMI with GA and HbA1c in Group II (Non-obese)

Table 3. HbA1c correlation with Lipid profile in Group I and group II

HbA1c vs	Group I (r)	p-value	Group II (r)	p-value
Triglycerides	-0.01	0.95**	0.248	0.12**
Total Cholesterol	0.16	0.32**	0.404	0.009***
HDL-cholesterol	-0.002	0.99**	0.468	0.0023***
LDL-cholesterol	0.17	0.29**	0.217	0.1786**

*** – Statistically highly significant
** – Statistically non-significant

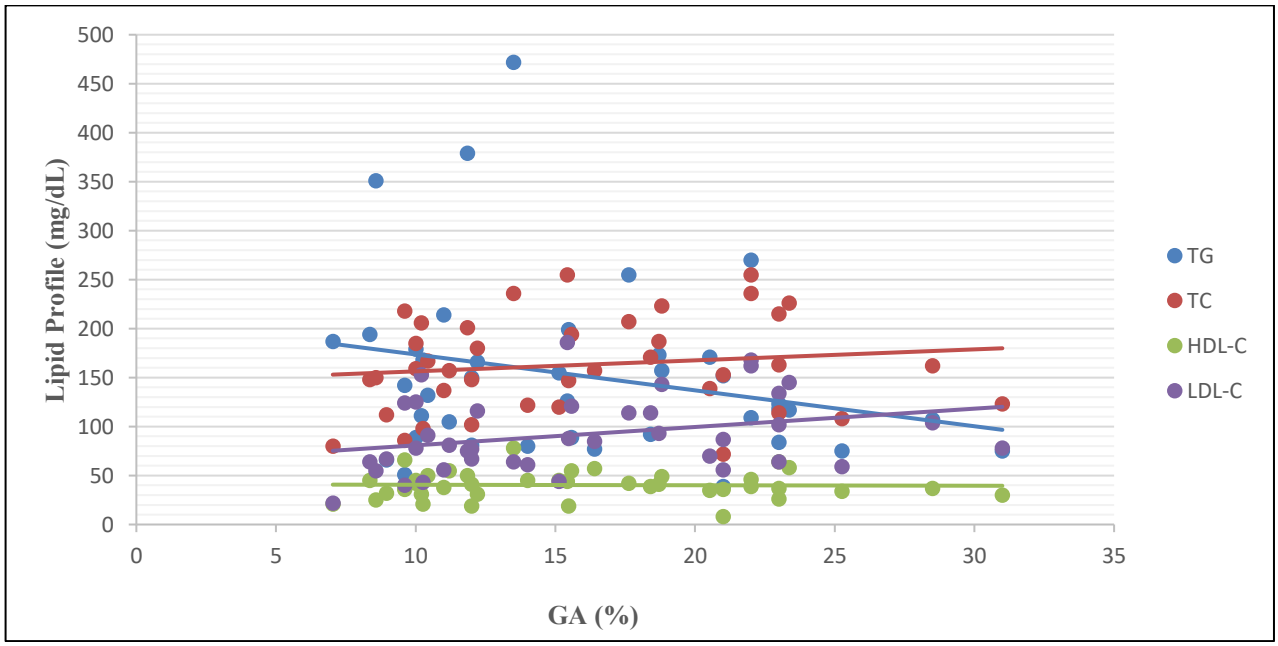

Figure 4: Graph showing the correlation of GA with Lipid Profile in Group II (Non-obese)

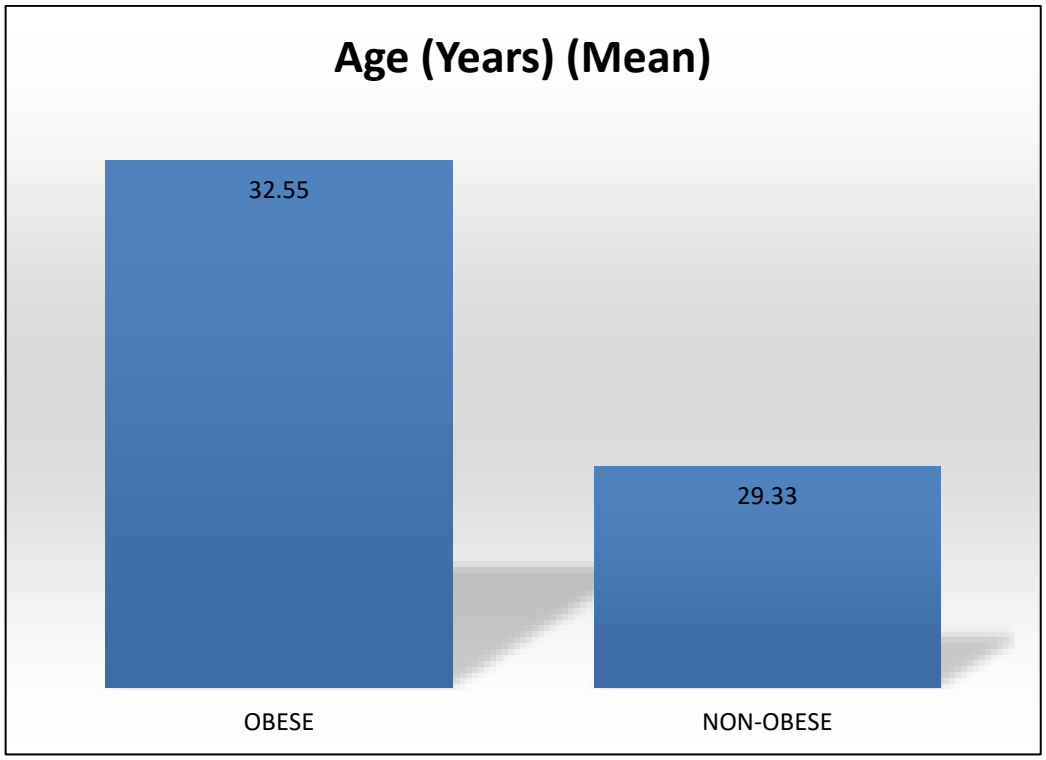

Figure 5: Diagram showing mean age in both the study groups

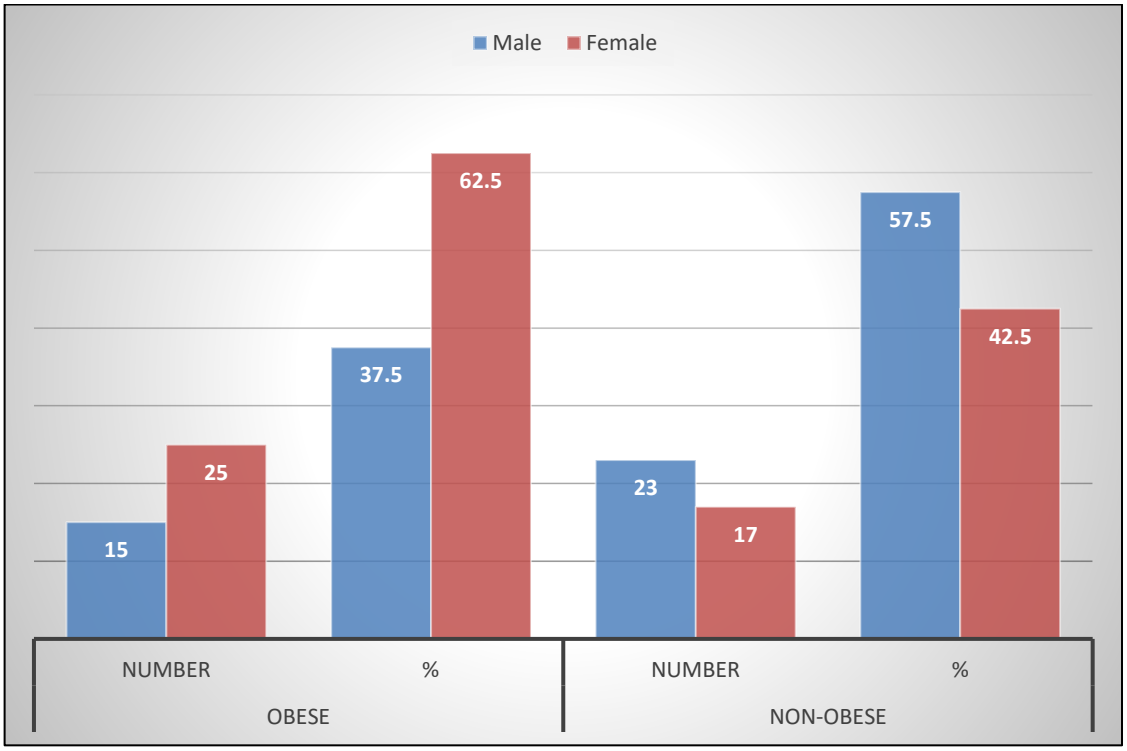

Figure 6: Diagram showing gender distribution in both the study groups

DISCUSSION

The biochemical basis of alterations of HbA1c in obesity is linked to the inflammation-induced development of insulin resistance. The expansion of adipose tissue causes a decrease in local oxygen supply, leading to cell-autonomous hypoxia with activation of cellular stress pathways. This causes cell-autonomous inflammation and the release of cytokines such as resistin, leptin and adiponectin causing inflammation and decrease insulin sensitivity. Hepatic inflammation can also occur in obesit, whereby inflammatory pathway activation could be the result of steatosis and/or increased hepatocyte stress pathway responses [19]. Glycated serum proteins (GSP) are formed by a non-enzymatic oxidation reaction that occurs upon binding blood glucose with plasma proteins, which are composed of 70% albumin.

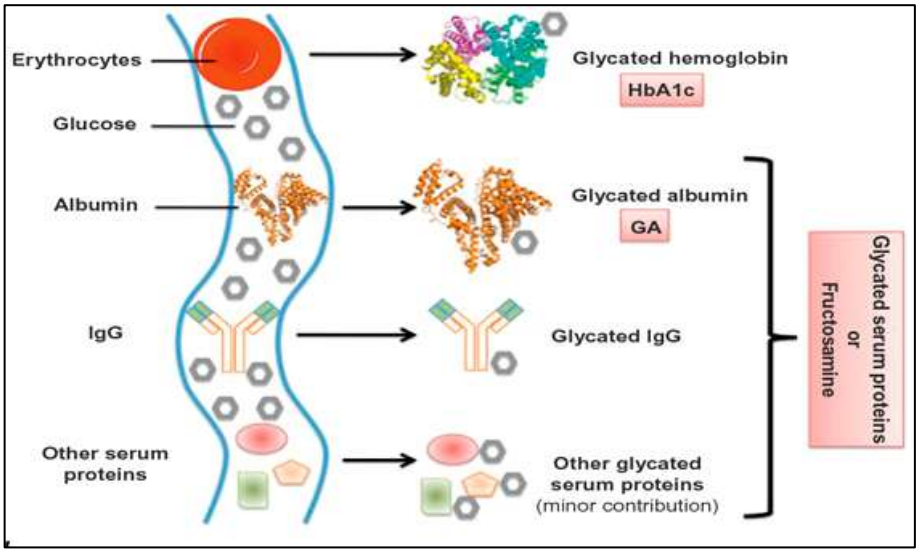

Figure 8: Glycation of serum proteins [20]

Albumin is the most abundant serum protein. GA is the main protein that is glycated in plasma, other glycated lipoproteins and globulin etc that may contribute to determining the total concentration of Fructosamine. In diabetes since glucose is high, levels of both Fructosamine and GA are high, therefore these can be used for assessing glucose control over a short to intermediate period of time. With respect to hemoglobin, whose life span in red blood cells is approximately 90-120 days, non-immunoglobulin serum proteins have a much lower half-life, approximately 14-21 days. This implicitly means that while HbAlc provides a long-term record of glycemic control (i.e., over a period of 2-3 months). The measurement of glycated plasma proteins e.g. GA, indicates glucose control of the previous 2 weeks. Also, when compared to hemoglobin, the rate of glycation of albumin is higher. [21]. Fructosamine is the investigation of choice to detect early dysglycemia in hemoglobinopathies, sickle cell disease, and anemia rather than HbA1c. In conditions where HbA1c fails to detect glycemic control, GA can be used as a reliable marker. GA indicates average blood glucose over 2-3 weeks. GA seems useful not only as an alternative index of glycemic control in conditions where HbAlc is unreliable, but also for identifying impaired control of blood glucose before any noticeable changes in HbAlc may occur [21]. A study has reported that the level of serum glycated albumin was significantly inversely associated with body mass index, body fat mass, and visceral adipose tissue. Furthermore, subjects with elevated body fat content have shown lower glycated albumin. Additionally, the results suggested that the inverse influence of obesity on albumin might be largely attributable to the effects of fat mass and visceral adipose tissue [22]. Another study conducted in the western Indian population to know the association of obesity with glycated hemoglobin stated that obesity is associated with increased amount of adipose tissue or its disproportionate distribution between central and peripheral body regions is related to dyslipidemia which causing an increase amount of hemoglobin glycation in non-diabetic subjects [23]. Research was conducted to derive the relationship between ratio of glycated albumin and HbA1c (GA/HbA1c) and body fat parameters, which reported that there is a significant decrease in GA/HbA1c ratio with increasing body mass index. Thus, GA/HbA1c was shown to be independently and negatively influenced by body mass index [24].

CONCLUSION

The finding from the present study supports that estimation of both GA and HbA1c can act as early detection marker for dysglycemia in healthy individuals. Higher value of HbA1c is associated with dyslipidemia in obese individuals.

Limitations of our study:

(1) Small sample size.
(2) Limited duration of time to complete the protocol.
(3) Insulin resistance and other hormonal factors affecting study group were not estimated which could have further supported the study findings.

Acknowledgement

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Ethical statement

The study was approved by institutional Ethics committee (BREC/Th/20/BIO/02, 2 April 2021, UHS, Rohtak) and conducted in accordance with Helsinki Declaration.

Conflict of interest
The authors have no conflict of interest to declare.

Authors contributions
All authors contributed to the study conception and design. Material preparations, data collection and analysis were performed. All authors have read carefully and approved the final manuscript.

Data availability statement
The data that supports the finding of the study are available with the author.

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