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Association Between Glycated Hemoglobin And Lipid Profile In Patients With Diabetic Nephropathy

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Abstract

Diabetes mellitus is a long term metabolic disorder distinguished by hyperglycemia and it is a chronic metabolic disorder characterized by hyperglycemia and disarrangement in metabolism of protein and fat. This study was done with 50 Diabetic nephropathy indoor and outdoor patients from April 2020 to June 2020 at Govt. Super Specialty Hospital, Trichy. Totally 50 cases were selected for this study males were 23 and female were 27. The total mean value of HbA1c in males was 9.20 ± 1.13 and in females mean HbA1c was 8.69±2.45. The diabetic nephropathy patients had HbA1c ≥ 8.0% with higher level of triglycerides, total cholesterol, LDL cholesterol and fasting blood glucose but remarkably HDL-cholesterol was decreased.

Keywords: Diabetic nephropathy, renal dysfunction, glycated hemoglobin, lipid profile.

Introduction

Diabetic nephropathy is the most severe problem in diabetes and the main usual source is final stage of renal disorder. Modern diabetic nephropathy is the major source of glomerulosclerosis and final stage of renal disorder globally. 20% to 40% of subjects with diabetes grow with this problem is not known (Odiliet al., 2011). The hypertension and diabetes combination is athreatened clinical situation that risk factors for both diabetes with individual or combined with micro and macro vascular risks of diabetes and diabetic linked mortality. Most of the diabetes will have hypertension and showed the 50% of cases had both diabetes and hypertension formed the seven times greater in mortality.

Glycated hemoglobin is a form of hemoglobin that is first used in the isolation of mean plasma glucose above a long duration of time, about 4-12 weeks. It is produced in a pathway of non enzymatic by normal hemoglobin display to increased plasma range of glucose. HbA1c test has been found in long term hyperglycemia than blood glucose (Aaron and vinik, 2001). The level of HbA1c higher than 7.0% has strong correlation with the formation of microvascular and macrovascular problems.

Glycated hemoglobin (HbA1c) is a marker of glycaemia and it is a forecaster of microvascular problem in diabetic subjects. So it is not clear that HbA1c is an index risk factor forthe complication of macro vascular in diabetic patients. It is the outcome of non-enzymatic reaction among glucose and hemoglobin amino groups. This type of reaction is called as glycosylation that involved in the proteins and mechanism via occurrence of glucotoxicity. Other mechanisms are oxidative stress, activation pathway of polyols, protein kinase-C activation, damage of endothelium, changes in hemodynamic and coagulation (Tophamet al., 2004).

The diabetes is established withHbA1c level ≥6.5% on two instances that is asymptomatic and symptomatic. The asymptomatic means the HbA1c levels increased to confirm the diagnosis (Roberto and Antonella, 2005). Both HbA1c levels must be higher than or equal to 6.5% of >48mmol/L and if the subject is symptomatic, the test for HbA1c is enough and the level noted is 48mmol/L. If the level of HbA1c lies between 42-47 mmol/mol of 5.7-6.4% then the subject is called as pre-diabetic, for those subjects the annualmonitoring of HbA1c is suggested.

Materials and Methods

Study Subjects:

The study was directed atGovt. Super Specialty Hospital, Trichy, in the Biochemistry department. Totally 50 cases were selected for this study,among that males were 23 and female were 27 who had minimal**≥5** year diabetes and maximal**≤5** year diabetes history were enrolled for this study. The study was conducted over three months of period from April 2020 to June 2020. The indoor and outdoor patients'departments were choose in the age group of 25-75 years.

All the 3 parameters estimation such as HbA1c, fasting blood glucose and lipid profile were performed using Architect c System and system of AEROSET with the help of special kits supplied by Hi media Laboratories.

1. The levels of HbA1c were analyzed by MULTIGENT HbA1c that is a 4 reagent kit supplied by Hi media Laboratories, Mumbai.

2. Blood glucose level in the blood was examined by single glucose reagent kit, ready-to-use supplied by Hi media Laboratories, Mumbai.

3. Lipid profile - The evaluation of dyslipidemia depends on the lipid parameters estimation in the fasting blood sample through serum triglyceride, total cholesterol, HDL- cholesterol, LDL- cholesterol was analyzed in samples of the patients. All these parameters were conducted on Architect c System and system of AEROSET with special kits.Serum Triglycerides, total cholesterol and HDL- cholesterol were examined by ready-to-use single reagent kits.

Specimen collection and preparation for analysis

Blood Collection- From the patients the fasting blood samples were collected in outdoor and indoor patient department of the hospital from morning 7 am to 10 am. Around 5ml of blood was collected and analyzed for three biochemical parameters like blood glucose, lipid profiles and HbA1c. The individual test tubes were needed at the collection period for each test of blood glucose, lipid profiles and HbA1c.

Serum separation- The serum was segregated from the blood and centrifuged and further it was used for estimation of blood glucose and lipid profiles. For testingglycatedHbA1c the whole blood was used.

Autoanalyzer: Architect c System and AEROSET System (USRDS, 2002**)**

The procedure, sampling, reagent delivery, mixing, processing and printing out the results were performed automatically by this Architect c System and AEROSET system. This system is a small rectangle shape electronic device that used to calculate the biochemical test' concentration.

The MULTIGEN HbA1c Immunoassay (NICE, 2005)

It was used to assay thepercent HbA1c in human blood quantitatively and measured the HbA1c concentration associated with total hemoglobin.

Principle

This immunoassay contains two divided concentration measurement namelyglycated hemoglobin and the Total Hemoglobin (THb). Both of the concentrations were used to determine the percent of glycated HbA1c. The total blood samples were initially pre treated with MULTIGENT Hemoglobin Denaturant. They lyzed the erythrocytes and degraded the hemoglobin by the pepsin enzyme to form the hemolysate. The concentration of glycated HbA1c was estimated from the same hemolysate.

Total Hemoglobin (THb)

TotheTHb reagent, the hemolysate was added and that contains alkaline solution for non-ionic surfactant. All of the hemoglobin was converted into hematin that formed green color solution. It was measured in the wavelength of 604nm.

HbA1c

TheHbA1c concentration was measured by immune turbidmetry using the method of micro particle agglutination inhibition. The antibody R1 of glycated HbA1c consists of special anti-HbA1c mouse monoclonal antibodies attached to microparticle. The agglutinator R2 of glycated HbA1c consists of many copies of haptens of HbA1c that covalently bound to the polymer. The increased rate of agglutination increased the absorbance. The absorbance was measured at 700 nm.

Reagents

Reagents Kit Details

MULTIGENT glycated HbA1c Reagent Kit is supplied as a liquid form, ready-to-use and it contains 4 reagent kits, turbidity method, manual testing procedure and the storage temperature is 2-8°C. The kit contains the following ingredients:

1. Thehemoglobin denaturant is pepsin in the concentration of 0.01%

2. R1 Total Hemoglobin (THb) is Sodium hydroxide with 0.4% of concentration

3. R1 HbA1c denaturant have the Micro particles coated with mouse antibodies to HbA1c with concentration of <0.1%

4. R2 HbA1c denaturant contains HbA1c hapten covalently attached to polymer - 2ng/ml.

Methodology

The reagents in the kit were prewarmed and the set the photometer (cuvette holder) to 37ºC. Dispense 1ml of hemolysis reagent into well labeled test tubes. Then add 20 µl of well mixed specimen sample and mix well. Let it stand for 5 minutes at room temperature and incubate at 37ºC until complete lysis was occurred. At the end ofthis period, read the absorbance at 600 nm. If the test cannot be carried out on the same day, the hemolysates was stored up to seven days at 2-8°C. For longer storage, the specimens were stored at -70°C for maximum 30 days.

Data analysis

All the analysis was performed using SPSS Version 20. Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean±standard deviation (SD) and results on categorical measurements are presented in number (%). Correlation coefficient was used to establish correlation.

Specimen collection and handling

Suitable specimens

Whole blood samples were collected using technique of standard venipuncture into glass or test tubes. The anticoagulants used are EDTA, heparin and lithium.

Percent HbA1c (NSGP)

The percentage of glycated HbA1c is the ratio of glycated HbA1c/THb with the conversion factor to associate with HPLC method.

Glycated HbA1c Fraction (IFCC)

The fraction of glycated HbA1c is the ratio of HbA1c/THb measured in mmol/L.

Results

The separate concentration measurement ofHbA1c was automatically performed using Auto-analyzer and measured in g/dl or mmol/L. The system was configured automatically to calculate the ratio of HbA1c to THb.

Conventional Units

The percent ofglycated HbA1c is produced by the equation that the calculation of the percent HbA1c is generated by using the following equation that comprised a factor to associate the glycated HbA1c results to HPLC method.

$$\frac{(\text{HbA1c (g/dl)} \times 100) - 3 + (0.2 \times \text{THb (g/dl)})}{\text{THb (g/dl)}} = \%\text{HbA1c}$$

For example, the HbA1c percent for a specimen consist of 1.000g/dl HbA1c and 1.40g/dl THb is calculated as:

$$\frac{(1.000\text{g/dl} \times 100) - 3 + (0.2 \times 14.0)\ \text{THb (g/dl)}}{14.0\text{G/dl}} = 6.9\%\ \text{HbA1c}$$

SI units

The glycated HbA1c fraction was calculated by the following equation.

$$\frac{(\text{HbA1c (mmol/L)} \times 1000\ \text{mmol/mol}}{\text{THb (mmol/L)}} = 52\ \text{mmol/mol HbA1c}$$

For example, glycated HbA1c mmol/L for a specimen containing 0.45mmol/L HbA1c and 8.68 mmol/L THb is calculated as

$$\frac{(0.45\ \text{mmol/L}) \times 1000\ \text{mmol/mol}}{8.68\text{mmol/L}} = 52\ \text{mmol/LHb1Ac}$$

Results and discussion

This investigation was carried out period of three months from April 2020 to June 2020.
The work of this research turns on quality of collection of data, gathered, arranged, extended, examined and dispersed. The data collected was used for diagnosis, prediction, investigation and analysis treatment.

Totally 50 diabetic nephropathy were selected for this research study. The participants' rate was 100%. Most of them were female participants at 27 of 54% while the male were 23 of 46%.The range of age of the patients were among 25 to 75 years had the increased frequency between 36-45 of 40% (Fig-1). The socio demographic of participant's gender were increased in female 27 (54%) compared to male 23 (46%) (Fig-2).

In this study, a constructive correlation of glycated hemoglobin (HbA1c) in diabetic nephropathy has been established. Glycated hemoglobin acts as a duplex biomarker for long duration glycemic control

and prognostic for diabetic nephropathy patients. The abnormal lipid profiles are common in diabetic patients and make diabetes susceptible to diseases of cardiovascular and atherosclerosis complications. Continuous increment of glucose level of blood i.e. hyperglycemia leads to protein glycosylation mainly cross linking of collagen and matrix of arterial wall. This is the slow process and caused endothelial cell dysfunction that contributes to the atherosclerosis (Melanie et al., 2003).

In this study, totally 50 diabeticnephropathy participants or patients were classified as male (23) and female (27). The mean range of glycated hemoglobin (HbA1c) was evaluated for male as 9.20 ± 1.13 and female as 8.69±2.45. The mean level of TC in male was 189.16± 44.12, in case of female participants were noted as 194.6±46.39. The TG mean value was ranged from 155.42± 61.38 and for female ranged from 185.33±75.19.

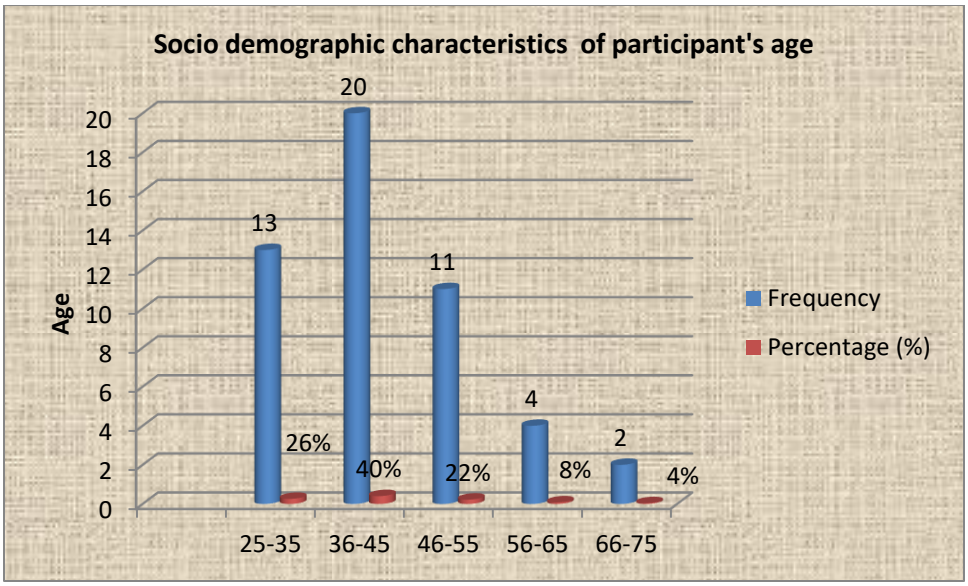
The elevated levels of free fatty acids into the liver having presence of sufficient amount of glycogen that stored in the liver assist production of triglyceride that induces the VLDL, apolipoprotein B and cholesterol secretion. This is due to disable ability of insulin to occupy the release of free fatty acid caused the higher formation of hepatic VLDL cholesterol that changed to increase the hepatic fat deposition (Vupputuri and Sandler, 2003).

In the case of diabeticnephropathy for LDL cholesterol, the mean value was estimated in male was 169.67±3.94 and 184.88±25.2 in female. For HDL cholesterol, the mean for male and female were evaluated as 41.18±1.95 and 41.60±8.97. Likewise, in FBG, the participants had the mean value ranged for male was 165.72±71.8 and female was 181.85±77.12 (Table-1). Diabetic nephropathy patients had increased level of HbA1c and lipid profiles are examined as a very high risk for cardiovascular diseases.

Table-1: Evaluation of Lipid profile parameters and HbA1c in the participants

S.No.	Total no. of Patients (n=50)	Males(n=23) Mean ±SD	Females (n=27) Mean ±SD
1	HbA1c (%)	9.20 ± 1.13	8.69±2.45
2	T.Cholesterol (mg/dl)	189.16± 44.12	194.6±46.39
3	LDL (mg/dl)	169.67±3.94	184.88±25.2
4	TG (mg/dl)	155.42± 61.38	185.33±75.19
5	HDL(mg/dl)	41.18±1.95	41.60±8.97
6	FBG (mg/dl)	165.72±71.8	181.85±77.12

Fig-1: **Socio demographic characteristics of participant's age**



The outcome results showed increased levels of lipid profiles in both genders but females had higher values that were found to have greater chance of cardiovascular diseases. So, the diabetic women have a chance to higher risk of mortality due to cardiovascular diseases. Women with diabetes were put through to the modification on the vascular function and other risk factors of cardiovascularissues than males. The lipid profile results indicates that the diabetic female patient had notably increased range of cholesterol, LDL, triglycerides, fasting blood glucose and HbA1c levels. This result was similar to the estimation by various scientists at different manners (Tonelli et al., 2006).

In this research study, the diabetic nephropathy participants were divided into two groups i.e. the first group consists of (n=32) males were 11 and females were 21 patients based on HbA1c cut of values ≥ 8.0% with poor glycemic control and second group consists of (n=18) males were 10 and females were 8 patients with HbA1c cut of values ≤ 8.0% fairly a good glycemic control. The results showed the comparison of the lipid profiles in group-I and group-II (Table-2). From the all lipid parameters the increased level of participants observed than the normal levels.

Table-2: Evaluations and Comparison of Lipid Profile results to the HbA1c

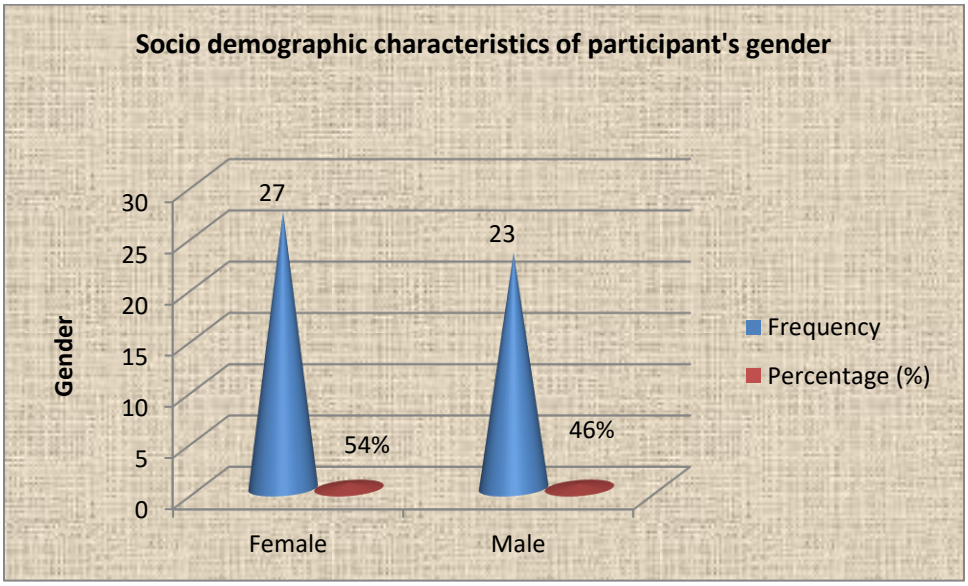
S.No.	Parameters	Glycated Hemoglobin (HbA1c); Mean ± SD	
		Group-I Poor glycemic control HbA1c ≥ 8.0 Subjects n- 32	Group-II Fairly good glycemic control HbA1c ≤ 8.0 Subjects n- 18

1	TG(mg/dl)	192.42 ±71.72	124.85 ± 36.07
2	T. Cho (mg/dl)	202.89 ±47.24	168.4 ± 33.64
3	LDL(mg/dl)	199.35±66.2	155.21±37
4	HDL (mg/dl)	37.66 ±7.08	43.54 ± 4.21
5	FBG (mg/dl)	201. 29 ±73.35	115 ± 17.55

Poor glycemic control -HbA1c ≥8.0 (n=32) Males 11& Females 21

Fairly good glycemic control – HbA1c ≤ 8.0% (n=18) Males 10&Females 8

Fig-2: Socio demographic characteristics of participant's gender



This study reports were compared to the study of (Vora et al., 2003) evaluated the higher prevalence of dyslipidemia in type 2 diabetic patients. Increased positive associations among HbA1c and FBG were found to be similar to various research works. Results of dyslipidemia and HbA1c were also similar to the study of (Strippoliet al., 2006).

The present study demonstrates that most of the diabetic patients had a poor glycemic control that affect the lipid profiles and lead to cause cardiovascular disease. It also confirmed the increased prevalence of dyslipidemia in patients withdiabetes than the non-diabetic patients. The higher level of HbA1c leads to the increment in the dyslipidemia to the diabetic patients. So, the diabetic participants have increased HbA1c and dyslipidemia can be reason for the higher risk of cardiovascular diseases. According to the study of (Sorkhouet al., 2003) the levels of HbA1c ≤8.0% caused the reduction of risk

factors of cardiovascular diseases. The improvement of glycemic control can decrease the risk of cardiovascular diseases within the proportions. The significant association among HbA1c and lipid profiles variations in both groups with (HbA1c ≥8.0%and ≤8.0%) denotes that the HbA1c can be indictedas the possible biomarker in the prediction of lipid profile variationin patients with diabetes nephropathy.

Conclusion

Thus, in a word, the HbA1c shows its associationwith lipid profile in diabetic nephropathy patients. The renal damage caused by this can be minimizedthrough early diagnosis would be useful to stop the possibility of the disease progress to final stage. Monitoring the levels of blood parameters analyzed in this study is easy, low cost and lesser time to access the diabetes.

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