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Glycosylated Hemoglobin in Young Healthy Kashmiri Adults for Reference Epidemiological Purposes and Prediction of the Risk of Future Diabetes

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Abstract: ***Background: Accumulation of glycated hemoglobin in the red cell reflects the average level of glucose to which the cell has been exposed during its life-cycle. Increased levels of glucose in the Hb contribute to more binding and consequently higher levels of glycosylated Hb. Glycated- Hb is also associated with a risk of diabetes and more strongly with the risk of cardiovascular disease and death from any cause as compared with fasting glucose. These data add to the evidence supporting the use of glycated- Hb as a diagnostic test for diabetes. Aims and objectives: Assessing the normal levels of HbA1c in young healthy adult Kashmiri population for reference and epidemiological purposes and prediction of the risk of future diabetes. Materials and Methods:This was a prospective study which was conducted in the department of physiology and department of Biochemistry, GMC Srinagar for a period of two years. The sample size included 500 normal, healthy adult Kashmiris of both sexes. Besides history, physical examination and routine investigations, HbA1c testing was done in these individuals by using an autoanalyser(ROCHE, HITACHI 912). Exclusion criteria included pregnant women, diabetics, individuals having any bleeding disorder and on any drugs like aspirin, steroids or dapsone. Statistical analysis was done by Two-sample t-test. Results: In our study males were more as compared to females(M=264, F= 236). The age group ranged from 20-60 years and the predominant age-group assessed was 20-30 years. The average HbA1c value obtained was more in males as compared to females, however less than the diabetic value, in the predominant age group assessed. There was a significant difference in the HbA1c values between males and females(p value 0. 008). Conclusion: HbA1c is a more comprehensive measure of total glucose exposure than fasting plasma glucose due to the representation of blood glucose in the post- prandial state in addition to the fasting state. HbA1c testing is a simple and effective investigation which can provide a baseline assessment of glycated hemoglobin in an individual and to reduce the delay in the diagnosis and treatment of hyperglycemic conditions with their complications.***

Keywords: Glycosylated/Glycated hemoglobin, HbA1c

1. Introduction

Glycated Hb- has shown to predict the progression from impaired glucose tolerance to diabetes mellitus. Advanced glycosylated end products have been shown to cross- link proteins (e. g. collagen, extra- cellular matrix proteins), accelerate athero- sclerosis promote glomerular dysfunction, reduce nitric oxide synthesis, induce endothelial dysfunction and alter extra cellular matrix composition and syndrome. The serum of advanced glycosylated end products correlates well with the levels of glycemia; these products accumulate as glomerular filtration rate declines[1, 2]. Glycated- Hb is also associated with a risk of diabetes and more strongly with the risk of cardiovascular disease and death from any cause as compared with fasting glucose. These data add to the evidence supporting the use of Glycated Hb as a diagnostic test for diabetes[3]. HbA_{1c} is a more comprehensive measure of total glucose exposure than fasting plasma glucose due to the representation of blood glucose in the post- prandial state in addition to the fasting state. Over the past several decades, diabetes mellitus has become a major health problem, world- wide, reaching epidemic proportions in many developing countries as well as in minority groups in the developed world[4].

2. Material and Methods

The present study was undertaken in the Department of Physiology, Government Medical College Srinagar to determine the levels of Glycosylated Hemoglobin in young Kashmiri adult population. All the subjects were personally interviewed to screen them for general information such as name, age, sex, address, occupation, personal habits and physical activity. General physical examination including body weight, height, BMI, waist-hip ratio was done. Physical activity level was assessed by nature of the work of the subject at the place of working (occupation) and from the life style. WHR interpretation was done as WHR >1 in men indicated abdominal fat accumulation. BMI interpretation was done by taking:

BMI < 25 as normal range,
BMI 25-30 as overweight (males), BMI > 30 as obesity (males)
BMI 25-28. 5 as overweight(females), & BMI ≥ 28. 6 as obesity (females).

From every subject 1. 5 mL of venous blood was collected after obtaining consent for the estimation of glycosylated

hemoglomin. The glycosylated hemoglomin was estimated by cation exchange resin method followed by steps:

Step 1— hemosylate preparation.

Step 2— glycohemoglobin separation and assay (GHb), the supernatant was pored into the cuvette for absorbance measurements at 415nm. A semiautomatic analyzer was used in the absorbance mode.

Step 3— total hemoglobin assay (THb) and followed by—

Step 4— calculations. The readings for unknown sample were recorded by the absorbance values.

$$\text{Glycohaemoglobin \%} = \frac{\text{Absorbance of glycohaemoglobin (GHb)}}{\text{Absorbance of total haemoglobin (THb)}} \times \text{Tf}$$

(Tf = Temperature factor for assay at 23^0C, Tf = 1. 0; at 30^0C, Tf = 0. 9).
Normal range of HbA_{1c} = 4. 5 t0 8. 0%

Five hundred subjects were included in the study and from them blood samples were taken for estimating HbA1c (%).

3. Results

In our study males were more as compared to females(M=264, F= 236). The age group ranged from 20-60 years and the predominant age-group assessed was 20-30 years. The average HbA1c value obtained was more in males as compared to females. There was a significant difference in the HbA1c values between males and females(p value 0. 008). The overall mean age ± standard deviation of the subjects was 34. 744±10. 569, of the males 35. 655±11. 218 and of the females 33. 725±9. 713. The overall mean value of BMI was 22. 74 with SD 3. 91, in males 22. 54±3. 7; whereas in females was 23. 18±2. 3. The mean WHR value was 0. 87, in males it was 0. 85 and in females 0. 83, with standard deviation of 0. 08, 0. 10 and 0. 05, respectively. The overall mean HbA1c value was 4. 566±0. 417. In case of males, the mean value of HbA1c was 4. 612±0. 382 and that for females was 4. 513±0. 447.

Table 1: The age and sex distribution of subjects.

TABLE 1

Descriptive Statistics	Age Distribution		
	MALES	FEMALE	TOTAL
N	264	236	500
Min	21	20	20
Max	60	60	60
A.M	35.655	33.725	34.744
S.D	11.218	9.713	10.569

Table 2: HbA1c levels in males and females.

TABLE 2

Descriptive Statistics	HbA1C		
	Male	Female	Total
N	264	236	500
Min	4.010	4.010	4.010
Max	5.290	5.190	5.290
A.M	4.612	4.513	4.566
S.D	0.382	0.447	0.417

Table 3: Age and sex distribution of individuals

TABLE 3
Age and Gender distribution of the Studied Subjects

Age (yrs)	Male		Female		Total		P value
	n	%	n	%	n	%	
≤ 30	126	47.7	107	45.3	233	46.6	0.041 (Sig)
31--40	49	18.6	80	33.9	129	25.8	
41--50	57	21.6	27	11.5	84	16.8	
> 50	32	12.1	22	9.3	54	10.8	
Mean ± SD (Max,Min)	35.7 ± 11.2 (21,60)		33.7 ± 9.7 (20,60)		34.7 ± 10.6 (20,60)		

4. Discussion

HbA1c levels depend on the blood glucose concentration. That is, the higher the glucose concentration in blood, the higher the level of HbA1c. Levels of HbA1c are not influenced by daily fluctuations in the blood glucose concentration but reflect the average glucose levels over the prior six to eight weeks. Therefore, HbA1c is a useful indicator of how well the blood glucose level has been controlled in the recent past and may be used to monitor the effects of diet, exercise, and drug therapy on blood glucose in diabetic patients[5, 6].

In healthy, non-diabetic patients the HbA1c level is less than 7% of total hemoglobin. It has been demonstrated that the complications of diabetes can be delayed or prevented if the HbA1c level can be kept close to 7%. In general, values should be kept below 8%[7]. Since HbA1c is not influenced by daily fluctuations in blood glucose concentration, it cannot be used to monitor day-to-day blood glucose concentrations and to adjust insulin doses nor can it detect the day-to-day presence or absence of hyperglycemia or hypoglycemia[8].

For decades, the diagnosis of diabetes was based on plasma glucose criteria, either the fasting plasma glucose (FPG) or the 2-h value in the 75-g oral glucose tolerance test[9]. In 2009, an International Expert Committee that included representatives of the ADA, the International Diabetes Federation (IDF), and the European Association for the Study of Diabetes (EASD) recommended the use of the

A1C test to diagnose diabetes, with a threshold of ≥6. 5%[10]. ADA adopted this criterion in 2010. HbA1c is associated with diabetes & its microvascular complications, macrovascular complications, risk of death and cardiovascular disease. The ultimate cause of microvascular and neuropathic complications is chronic hyperglycemia. Intracellular hyperglycemia activates the enzyme aldose reductase which increases the formation of sorbitol in the cells which in turn reduces cellular Na-K ATPase. Hence Glycated- Hb is widely used for monitoring long term glycemic control in known diabetic patients and the prognostic value of glycated Hb has been investigated, in study of patients with normal glucose tolerance[11, 12].

HbA1c is measured as the ratio of glycosylated to nonglycosylated hemoglobin. Measurement of HbA1c is accepted as a useful index of mean blood glucose in the treatment of patients with diabetes. Decision regarding treatment is often based on HbA_{1c}[13]
.
There is growing interest in the blood glucose concentrations because glucose reacts with amino groups of plasma and tissue proteins (Amadori reaction) to form glycated proteins depending on blood glucose concentrations. These glycated proteins gradually transform non-enzymatically into advanced glycated end-products and have been reported to result in altered protein functions of the affected molecules. Both micro and macro vascular complications and increased atherosclerotic risk were reported to be associated with advanced glycation end-products. Research on blood glucose concentrations was facilitated by the identification of glycated haemoglobin (HbA_{1c}) as a biomarker of long term glucose homeostasis[14].

Hyperglycemia is an independent risk factor for cardiovascular disease, with no apparent threshold. In clinical practice, glycemic exposure is measured by haemoglobin A_{1c} (HbA_{1c}) levels. These values reflect the contribution of fasting and postprandial plasma glucose levels during the previous 2 to 3 months. Epidemiologic studies have indicated that haemoglobin A_{1c} levels (5. 5%) were already associated with a substantially increased risk for cardiovascular mortality when he fasting plasma glucose levels were generally normal (Gerich, 2006). Furthermore, glucose concentrations play an important role in the metabolic syndrome. High serum glucose concentrations indicate the beginning of or existing glucose intolerance and insulin resistance, which may result in Type 2 diabetes (Hannah and Howard, 1994). In the presence of hypergycemia, the process of glycation may also contribute to the pathogenesis of disease through accumulation of advanced glycosylation end-products (AGE) which may adversely affect vascular and neural function (Brownlee, 2000)[15, 16].

In our study males were more as compared to females(M=264, F= 236). The age group ranged from 20-60 years and the predominant age-group assessed was 20-30 years. The average HbA1c value obtained was more in males as compared to females. There was a significant difference in the HbA1c values between males and females(p value 0. 008). The average HbA1c level in males was 4. 612 and in females it was 4. 513, which is associated with a lower risk of diabetes mellitus.

5. Conclusion

HbA1c is a more comprehensive measure of total glucose exposure than fasting plasma glucose due to the representation of blood glucose in the post- prandial state in addition to the fasting state. HbA1c testing is a simple and effective investigation which can provide a baseline assessment of glycated hemoglobin in an individual and to reduce the delay in the diagnosis and treatment of hyperglycemic conditions with their attendant complications. In our study HbA1c levels were more in males as compared to females, but higher levels of HbA1c are less common in young individuals and hence the risk of diabetes is less, especially in those without any risk factors for diabetes.

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