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GLYCOSYLATED HAEMOGLOBIN IN NORMAL HEALTHY SUBJECTS WITH AND WITHOUT FAMILY HISTORY OF DIABETES MELLITUS- A HOSPITAL BASED STUDY

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ABSTRACT
Glycated hemoglobin is produced by non-enzymatic condensation of glucose molecules with free amino groups on the globin component of hemoglobin and the major form of glycated hemoglobin is termed hemoglobin A1c (HbA1c), which normally comprise only 4-6% of total hemoglobin. In the presence of hyperglycemia, 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. The serum level of AGEs correlates with the level of glycemia and these products accumulate as glomerular filtration rate declines. A total of 200 normal subjects were taken. Body mass index was calculated by Quetelet's Index and waist-hip ratio was taken in percent. Dietary history along with physical status of the body was taken and glycosylated haemoglobin (HbA_{1c}) was estimated. The overall mean HbA_{1c} value was found to be 7.26 ± 1.41. In case of males, the mean HbA_{1c} value was 7.28 ± 1.38 and in females it was 7.04 ± 1.4. As age increased from one decade to another, statistically no significant effect was seen on glycosylation of haemoglobin. As WHR increased from 0.8 to 1 (which interprets abdominal obesity), the glycosylation of haemoglobin also increased. Statistically significant ($P< 0.05$) relationship between place of residence and glycosylation of haemoglobin was observed among the subjects of urban background as compared to subjects of rural background. A significant relationship was observed in subjects who consumed > 2501 calories/day and had positive family history of diabetes mellitus with glycosylation of haemoglobin. Statistically significant relationship existed between intake of alcohol and glycosylation of haemoglobin with positive family history of diabetes mellitus. Smoking also had significant effect on glycosylation of haemoglobin ($P< 0.05$). Highly significant relationship was observed in smokers with family history of diabetes mellitus. Non significant relationship was found between physical activity level and glycosylation of haemoglobin in subjects with or without family history of diabetes mellitus.

KEYWORDS: Glycosylated Hemoglobin, HbA1c, Hyperglycemia, Diabetes mellitus.

INTRODUCTION
Glycated hemoglobin is produced by non-enzymatic condensation of glucose molecules with free amino groups on the globin component of hemoglobin and the major form of glycated hemoglobin is termed hemoglobin A1c (HbA1c), which normally comprise only 4-6% of total hemoglobin. The remaining glycated hemoglobin (2-4%) consists of phosphorylated glucose or fructose and is termed hemoglobin A_{1a} (HbA_{1a}) and hemoglobin A_{1b} (HbA_{1b}).[1] Many proteins are known to react with carbohydrates at the peptide N-terminus forming glycosylated peptides and glucose can rapidly react with hemoglobin to form a labile aldimine (Schiff base).[2] A considerable decrease in the values for glycosylated hemoglobin as measured by cation-exchange chromatographic methods is found in subjects with hemoglobin S or hemoglobin C. This decrease is proportional to the percentage of the abnormal hemoglobin.[3] In the presence of hyperglycemia, 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.[4] GEs have been shown to cross-link proteins *(e.g.,* collagen, extracellular matrix proteins), accelerate atherosclerosis, promote glomerular dysfunction, reduce nitric oxide synthesis, induce endothelial dysfunction and alter extracellular matrix composition and structure. The serum level of

AGEs correlates with the level of glycemia and these products accumulate as glomerular filtration rate declines.[5] The DCCT demonstrated that improvement of glycemic control reduced non-proliferative and proliferative retinopathy (47% reduction), micro-albuminuria (39% reduction), clinical nephropathy (54% reduction), and neuropathy (60% reduction). Improved glycemic control also slowed the progression of early diabetic complications. The result of the DCCT predicted that individuals in the intensive diabetes management group would gain 7.7 additional years of vision, 5.8 additional years free from end-stage renal disease (ESRD) and 5-6 years free from lower extremity amputations. If all complications of hyperglycemia were combined, individuals in the intensive diabetes management group would experience 15.3 more years of life without significant microvascular or neurologic complications of diabetes mellitus, compared to individuals who received standard therapy.[6] The purpose of this study was to determine and to assess the effects of diet, alcohol, cigarette smoking and level of physical activity on glycosylated haemoglobin (HbA_{1c}) in normal healthy subjects with and without family history of diabetes mellitus.

MATERIALS AND METHODS

The present study was carried out in the Postgraduate Department of Physiology, Government Medical College Jammu with equipment facility provided by the Postgraduate Department of Pharmacology and Therapeutics Government Medical College Jammu. The period of study was of one year. After obtaining approval of the Institutional Ethics Committee and written informed consent from the subjects, a total of 200 normal subjects were taken. The subjects were the attendants of the patients attending the Outpatient Department of Endocrinology/Medicine and attendants of Indoor patients of Medicine Wards of the Government Medical College, Jammu.

Anthropometric parameters

Body mass index (BMI)

Body mass index was calculated by Quetelet's Index i.e., Weight (kg)/ Height $(m)^2$.[7]

Waist-hip ratio (WHR)

It was measured as waist-hip ratio and taken in percent. WHR interpretation:
WHR > 1.0 in men Indicated abdominal fat accumulation. WHR >0.85 in women indicated abdominal fat accumulation.[8]

Dietary history

From the subjects enquiries were made retrospectively about the nature and quantity of food eaten during last 24 hours by oral questionnaire method. The caloric value of cooked, uncooked food and beverages was calculated as per the guidelines of Indian Council of Medical Research.[8]

Physical activity level (PAL)

Those subjects who performed physical activities like weight lifting, digging or fast bicycling for at least 30 minutes at a time for 4-5 days per week were categorized under 'vigorous physical activity'. Those subjects who performed activities like carrying light loads, bicycling or brisk walking at a regular pace or playing a game like tennis, badminton for at least 30 minutes at a time for 4-5 days per week were categorized under 'moderate physical activity'. Those subjects who performed activities like walking at work and at home, for recreation or leisure for 4-5 days per week were categorized under 'light physical activity'. Those subjects who spent most of the time sitting while at work, at home or during leisure time, this includes time spent sitting at a desk, visiting friends, reading, travelling on a bus or personal car or sitting down or lying down to watch television were categorized under 'sedentary physical activity'.

Personal habits

History of smoking

The history of smoking was taken from the subjects.

History of alcohol intake

The history of alcohol intake was taken from the subjects.

Estimation of glycosylated haemoglobin (HbA_{1c})

The glycosylated haemoglobin was estimated by cation exchange resin method using glycohaemoglobin reagent kit procured from Vector Bioteck Pvt. Ltd., India.

Normal range of HbA_{1c} as per kit was 4.5 to 8.0%.

A semiautomatic analyzer (ERBA Chem Pro. Inc., Transasia Biomedicals Ltd., India) was used for the estimation of glycosylated haemoglobin (HbA_{1c}).

Statistical analysis

Analysis was performed using computer software Excel. One-way analysis of variance (ANOVA) and student‘t’ test was used to compute statistical inference. A P value of < .05 was considered statistical significant.[9]

RESULTS

The study was conducted to find out the relationship of age, sex, place of residence, obesity particularly abdominal obesity (BMI and WHR), lifestyle influence i.e., cigarette smoking, intake of alcohol, physical activity level as well as the total calories intake per day with the glycosylation of haemoglobin in normal healthy subjects with and without family history of diabetes mellitus, thereby depicting the level of hyperglycemia. The various baseline characteristics of the subjects are presented in Table I. The age of the subjects ranged from 20 to 79 years. There were 164 males and 36 females, comprising 82% and 18%, respectively. The demographic distribution profile

included 117 subjects (58.5%) of rural origin and 83 (41.5%) subjects of urban origin. The 30% (n = 60) subjects were alcoholics and 70% (n = 140) were non-alcoholics. The distribution of the subjects according to their alcohol intake is given in Table II. There were 19% (n = 38) subjects with family history of diabetes mellitus and 81% (n = 162) without family history of diabetes mellitus. From the table III, the data showed more glycosylation of haemoglobin in the age groups of 20-39 years and 60-79 years than in the age group of 40-59 years. The males showed higher level of glycosylation as compared to females. The subjects of urban background showed mean HbA_{1c}value of 7.74 with standard deviation of 1.39. The subjects of urban background had significantly higher value of HbA_{1c} as compared with rural subjects (Table IV). The Table V depicts that as the BMI increases from 25 to > 30, there was no increase in the glycosylation of hemoglobin. Table VI shows relationship of WHR with mean HbA_{1c}value. The data showed as WHR increased, the glycosylation of haemoglobin also increased from 7.04 ± 1.06 to 7.54 ± 0.95.The overall result from the data depicted in (Table IX, X, XI) was subjects who smoke and have family history of diabetes mellitus had higher glycosylation of hemoglobin. In overall subjects who did not smoke and neither had family history of diabetes mellitus, glycosylation of hemoglobin was less (67.5% subjects with < 8% HbA_{1c}). From table XII, the result depicts that physical activity level had no effect on glycosylation of haemoglobin.

Table I: Various baseline characteristics of the subjects.

parameters	Mean ±SD		
	Overall	Male	Female
Age	39.18±12.58	40.06±14.16	35.19±10.77
BMI	22.74 ±3.91	22.54 ±3.7	23.18 ±2.3
WHR	0.87 ±0.08	0.85 ±0.10	0.83 ±0.05
Calories intake/day	1772.86 ±509.46	1856.92 ±512.83	1456.80 ±274..51
HbA_{1c}	7.26 ±1.41	7.28 ±1.38	7.04±1.4

Table II: Showing number of subjects according to their history of alcohol intake.

Alcohol intake	Total	
	n	(%)
Yes	60	(30)
No	140	(70)
Total	**200**	**(100)**

Table III: Relationship of age with mean HbA_{1c} value.

Age (years)	Mean HbA_{1c}±SD	Statistical inference	
20 – 39 (n = 96)	7.37 ± 1.37	t =1.57	'NS'
40 – 59 (n = 89)	7.05 ± 1.41	t =0.65	
60 – 79 (n = 15)	7.30 ± 1.08	t = 0.26	

n = Number of subjects 'NS' = Non – significant
t = student 't' test

Table IV: Relationship of place and residence with mean HbA_{1c} value.

Place of residence	Mean HbA_{1c}± SD	statistical inference	
Rural (n= 117)	7.09 ± 1.42	t= 1.99	'S' (p< 0.05)
Urban (n = 83)	7.74 ± 1.39		

n = Number of subjects 'S' = significant

Table V: Relationship of BMI with mean HbA_{1c} value.

BMI	Mean HbA_{1c}± SD	Statistical inference	
< 25 (n=33)	7.30 ± 1.39	F = 1.22	'NS'
25- 30 (n=30)	7.30 ± 1.49		
>30 (n= 137)	7.33 ± 1.29		

n = Number of subjects 'NS'= Non – significant F = ANOVA.

Table VI: Relationship of WHR with mean HbA_{1c} value.

WHR	Mean HbA 1c ± SD	Statistical inference	
< 0.8 (n=25)	7.04 ± 1.06	F = 3.89	'S' (P < 0.05)
0.8 – 1 (n=156)	7.18 ± 1.46		
> 1 (n= 19)	7.54 ± 0.95		

n = Number of subjects 'S'= significant F = ANOVA.

Table VII: Relationship of alcohol with HbA_{1c} (%).

Alcohol intake	HbA_{1c} (%)					
	<8		>8		Overall	
		%		(%)		(%)
Yes	7	(31.3)	13	(26.0)	60	(30.0)
No	0.3	(68.7)	37	(74.7)	140	(70.0)

Chi – square = 0.50 OR = (CI 0.45, 1.55) P = 0.47.

Table VIII: showing relationship of alcoholics with HbA_{1c} (%) in normal healthy subjects with and without family history of diabetes mellitus.

Family H/o of Diabetes mellitus	HbA_{1c} (%)			
	<8		>8	
	N	(%)	n	(%)
Yes	9	(19.1)	4	(30.8)
No	38	(80.9)	9	(69.8)
Total	47		13	

OR = 1.88 (CI 0.42, 7.46).

Table IX: showing relationship of smoking with HbA_{1c} (%).

Smoking	HbA_{1c} (%)					
	<8		>8		Overall	
	n	%		(%)		(%)
Yes	46	(32.4)	8	(48.3)	4	(37.0)
No	96	(67.6)	0	(74.7)	26	(63.0)

Chi – square = 4.46 OR = 1.96 (CI 1.04, 3.59).

Table X: Showing relationship of smoking with HbA_{1c} (%) in normal healthy subjects with family history of diabetes mellitus.

Smoking	HbA_{1c} (%)			
	<8		>8	
	n	(%)	n	(%)
Yes	8	(32.0)	10	(76.9)
No	17	(68.0)	3	(23.1)
Total	25		13	

OR = 7.08 (CI 1.53, 32.13).

Table XI: Showing Relatioship of smoking with HbA_{1c} (%) in normal healthy subjects without family history of diabetes mellitus.

Smoking	HbA_{1c} (%)			
	<8		>8	
	n	(%)	n	(%)
Yes	38	(32.5)	18	(40.0)
No	79	(67.5)	27	(60.0)
Total	117		45	

OR = 1.38 (CI 0.67, 2.80).

Table XII: Showing relationship of physical activity level with HbA_{1c} (%).

Physical Activity level	HbA_{1c} (%)			
	<8		>8	
	N	(%)	n	(%)
Vigorous*	27	(18.0)	13	(26.0)
Moderate	54	(36.0)	25	(50.0)
Light	48	(32.0)	9	(18.0)
Sedentary	21	(14.0)	3	(6.0)
Overall	**150**		**50**	

*Reference Chi – square = 7.64.

Table XIII: Showing Realtionship of intake of calories / day (>2501 with HbA_{1c} (%) in normal healthy.

Family H/o of Diabetes mellitus	HbA_{1c} (%)			
	<8		>8	
	N	(%)	n	(%)
Yes	2	(14.3)	2	(40.0)
No	12	(85.7)	3	(60.0)
Total	14		5	

OR = 4.00 (CI 0.42, 40.85).

DISCUSSION

There is a 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 glycation end-products and have been reported to result in altered protein function of the affected molecules. Both micro and macrovascular 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. Epidemiologic studies have indicated that haemoglobin A_{1c} levels (5.5%) were already associated with a substantially increased risk for cardiovascular mortality when the fasting plasma glucose levels were generally normal.[2] So the present study was undertaken to find out the relationship of diet, alcohol intake, cigarette smoking and physical activity level with glycosylation of haemoglobin in normal healthy subject with and without family history of diabetes mellitus.

Age, sex and glycosylation of haemoglobin

In our study, the distribution of HbA_{1c} was approximately same with a slight difference between mean and standard deviation values at all ages and was statistically non-significant (Table III). The largest elevation of glycohaemoglobin occurred in the age group of 20-39 years age group. In our study, the overall mean value of glycosylated haemoglobin was 7.26% (Table I). Statistical inference between the males who had a mean glycosylated haemoglobin value of 7.28% as compared to females 7.04% was non-significant.[23,6,12,30]

BMI, WHR and glycosylation of haemoglobin

In the present study, the BMI did not show a statistically significant relationship with mean value of glycosylated haemoglobin. Further, increasing BMI had no effect on mean value of glycosylated haemoglobin.

In the present study, statistically significant relationship existed between mean value of HbA_{1c} (%) and waist-hip ratio ($P < 0.05$). HbA_{1c} increased with increasing WHR (Table VI). Similar results have been depicted by other studies (10-14).

Places of residence and glycosylation of haemoglobin

Urban population are said to be exposed to a large number of non communicable diseases because of adoption of western lifestyle, which have direct as well as confounding impact on glucose metabolism and insulin sensitivity. Recent epidemiological data showed the prevalence of diabetes in India is 8-10%.

About 2.4% of rural population and 8.4% of urban population is affected by diabetes.In the present study, the mean HbA_{1c} value in urban populance was 7.74% and mean HbA_{1c} value in rural populance was 7.09% (Table IV). The relationship between place of residence and glycosylation of haemoglobin was statistically significant (< 0.05). These findings might support the hypothesis that urbanization influence glycation of haemoglobin (HbA_{1c}).[6,15-16]

Effects of diet (calories/day), alcohol, cigarette smoking and physical activity level on HbA_{1c} (%) with and without family history of diabetes mellitus.

Diet and glycosylated haemoglobin

In the present study, the total calorie intake/day was within normal range. It ranged between 1001-2501 calories and showed no relation with glycosylation of haemoglobin. This could be due to higher consumption of

wheat grains in the form of "chapatties". Significantly greater ORs were found for the highest category of energy intake (OR = 4.00 when consumption of energy was > 2501) (Table XIII). It was four times than subjects with low category of energy intake.[17,18,12,1,19,20]

Alcohol and glycarylated haemoglobin

In the present study, overall there was no statistically significant relationship between intake of alcohol and HbA_{1c} (%) (Table VII). However, high OR (1.88) was observed in non-diabetic' individuals with positive family history of diabetes mellitus (Table V Ill).[8,12,21,22,23]

Smoking and glycosylated haemoglobin

In our study, the relationship between smoking and glycosylation of haemoglobin was statistically significant ($P < 0.05$).Further, in smokers with family history of diabetes mellitus OR was in highest range (7.08) (Table X) as compared to smokers without family history of diabetes mellitus (OR = 1.38) (Table XI). It was revealed that smoking with positive family history of diabetes mellitus increases the glycosylation of haemoglobin by 7.08 times.[24,25,14,12]

Physical activity level and glycosylated haemoglobin

In the present study, the relationship between physical activity level and glycosylation of haemoglobin was statistically non-significant. The study further revealed that family history of diabetes mellitus and physical activity level had no effect on glycosylation of haemoglobin.[26,20,22,15,27]

CONCLUSION

The present study on normal healthy subjects revealed that the overall mean $HbA_{1c} \pm SD$ was 7.26 ± 1.41. In males, mean $HbA_{1c} \pm SD$ was 7.28 ± 1.38 and in females it was 7.04 ± 1.40. The normal range of HbA_{1c} is 4.5 to 8.0%. Above findings suggested that HbA_{1c} (%) even in normal subjects were on higher side. The gender showed no statistically significant difference in the glycosylation of haemoglobin, although females showed a bit lower value of HbA_{1c} as compared to males. As age increased from one decade to another, statistically no significant effect was seen on glycosylation of haemoglobin. Statistically significant ($P < 0.05$) relationship between place of residence and glycosylation of haemoglobin was observed among the subjects of urban background as compared to subjects of rural background. This could be due to more stressful life in urban setup. No significant change was observed in the mean value of glycosylation of haemoglobin among normal healthy subjects with change in BMI from 25-30. A statistically significant relation was observed as WHR increased from 0.8-1 (which interprets abdominal obesity), the glycosylation of haemoglobin also increased. A significant relationship (OR = 4.00; CI 0.42, 40.85) was observed in subjects who consumed > 2501 calories/day and had positive family history of diabetes mellitus with glycosylation of haemoglobin. Statistically significant (OR = 1.88; CI 0.42, 7.46) relationship existed between intake of alcohol and glycosylation.

Competing Interests

The authors declare that there were no competing interests.

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