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TO STUDY THE RELATIONSHIP BETWEEN THE GLYCOSYLATED HAIR PROTEIN AND HBA1C FOR ASSESSMENT OF BLOOD GLUCOSE LEVEL IN DIABETES MELLITUS TYPE II

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ABSTRACT

BACKGROUND: Hair is a dead tissue and is composed of keratin and related proteins. Hair is a protein of few growing cells at the base of the hair root. If the patient is diabetic glycosylation occurs in these growing cells.Since the extent of glycation of hair protein varies with the distance from the hair root, we hypothesized that glycation of hair protein might provide insight into blood glucose level over a period of several months to one year.So hemoglobin A1c (HbAIc) may be established as an indicator of long-term blood glucose control in diabetic patients. It is formed by non-enzymatic reaction of glucose with Hb.The objective of this study was to estimate the furosine(an intermediate in the breaking down of amadori compounds like glycosylated proteins) level by HPLC of glycosylated hair proteins, to find interrelation of fasting glucose with HbA1c and furosine in diabetic patients, to determine the efficacy of Glycosylation of HbA_1c and furosinemay be a reliable marker in diabetic patients.

STUDY DESIGN: This study was carried out in the department of Biochemistry Basic Medical Sciences Institute (BMSI) Jinnah Postgraduate Medical Centre Karachi.

METHODS: A total of 200 subjects were included in the study. 100 diagnosed type II and 100 control normal subjects. Hair from the scalp 12 cm long and 50 mg in weight was collected from each subject to measure furosine level. Furosine level was determined by HPLC and HbA1c, FBSand serumprotein by enzymatic kit method.

RESULTS: When the mean values were compared with that of control it depicted significantly high level (0.001) of furosine, HbA1c and FBS.

CONCLUSION: The furosine, HbA1c and FBS levels increases in diabetic patients while total serum protein were unaffected. Weight and BMI increases in diabetic but height was unaffected.

KEY WORDS: Diabetes mellitus type II, furosine, HbA1c, fasting blood sugar, glycation of hair protein

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia due to absolute or relative deficiency of insulin, impaired action of insulin or both[1]. Diabetes occurs when pancreas does not produce enough insulin or body cannot effectively use the insulin it produce[2]. Pakistan has the 4th rank in highest diabetic population countries[3].To prevent the complication in diabetes mellitus the glucose level in diabetics must be monitored for a long time. For this purpose, analysis of glycated protein in hair makes possible to evaluate past advanced stage of diabetes mellitus[4]. In the present study we were attempting to find the relationship between the glycosylated hair protein and HbA1c.

Hair is a protein filament that grows through the epidermis from follicles deep within the dermis, found exclusively in mammals. It is one of the defining characteristic of the mammalian class. Typical mammalian hair consists of the shaft, protruding above the skin and the root which is sunk in a follicle or pit beneath the skin surface. Hair is a protein of few growing cells at the base of the root. Hair is a dead tissue and is composed of keratin and related proteins. The hair follicle is a tube like pocket of the epidermis that encloses a small section of the dermis at its base. Human hair is formed by rapid divisions of cells at the base of the follicle. As the cells are pushed upward from the follicle's base, they harden and undergo pigmentation[5].

Glycosylation in the scalp hair protein remains stable along the length of hair from scalp to tip in normal subjects and suggests that a sufficiently long hair sample may offer a long term record of degree of hyperglycaemia. This may be useful in the investigation of hyperglycaemia and microvascular complications of diabetes mellitus[3]. Hair glycation may serve as a valuable indicator both of long term blood glucose trends and of the relationship between diabetic complications and blood glucose[6].

Glucose sticks to the hemoglobin to make a glycosylated hemoglobin molecule, called hemoglobin A1C or HbA1C. The glycosylation proceeds by non-enzymatic reaction (Maillard reaction) in red blood cells. This glycosylation is proportional to the blood glucose level, the higher the blood glucose level the faster will be the formation of HbA1c. The life span of RBC is 60-120 days but the HbA1c level is in part affected by the elevated blood glucose level over a period of only three months. A normal non-diabetic HbA1C is 3.5-5.5%.HbAIc is established as an indicator of long-term blood glucose control in diabetic patients[7].

Similar glycosylation has been reported to occur in tissue proteins such as hair, lens, collagen and glomerular basement membrane, lipoprotein or hormone. Moreover analysis of these tissue proteins can acknowledge the condition of glucose control for a particular time and speed of growth of the protein. Meanwhile, acid hydrolysis of an Amadori compound, fructose-lysine; produces furosine[8].

Furosine a by-product generated by acid hydrolysis of protein in hair has been proposed as a marker compound for glycation of protein. Among several organic tissues, hair obtained non-invasively was expected to provide information about glycated protein levels over a period of time[9].Furosine derived from fructose-lysine formed by binding of lysine residue of hair protein can be measured and used as an indicator of glycation. It has been postulated that the past blood glucose level at arbitrary time can be estimated from hair glycation by considering the rate of hair growth and the distance of hair from the scalp[10]. Traditionally, the most sensitive and specific determination of non-enzymatic protein glycation has involved an 18-24-h acid hydrolysis in order to generate the compound furosine, which has been detected employing reversed-phase HPLC[11].

MATERIAL AND METHODS

This study was carried out in the Department of Biochemistry Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre Karachi; in collaboration with the Medical Ward JPMC Karachi.

A total of 200 subjects of both sexes were included in this study. 100 subjects of age above 40 years suffering from diabetes mellitus type-II were taken from Diabetic clinic, Department of Medicine, JPMC, Karachi and 100 age matched normal healthy control subjects were selected from general population for comparison. The subjects were interviewed in detail regarding the name, age, address, occupation, medical and surgical history and any other associated chronic illness on the questionnaire/proforma. Patients with anemia, liver diseases, renal disease, thyroid and or growth hormone abnormalities, pregnant women were excluded from the study.

Blood samples were collected for HbA1c, FBSandserum protein lipid. Furosine level was determined by HPLC and HbA1c, FBSand serum

protein by enzymatic kit method.The hair furosine was determined by high performance liquid chromatography (HPLC) method.

DETERMINATION OF HAIR FUROSINE

The hair furosine was determined by high performance liquid chromatography (HPLC) method[13].

Hair samples 12cm long,12cm proximal from the scalp, was collected from each diabetic and control subjects. Hair samples taken from parietal or temporal areas was washed first in petroleum ether and then with an ultrasonic washing instrument for furosine determination. The washed hair samples were cut into as many small pieces as possible. Fifty milligrams of the sample was hydrolyzed in 2 ml of 6 Normal HC1 at 95°C for 30 h and evaporated to dryness. The sample was reconstituted in I ml distilled water. The solution was passed through a membrane filter (Millipore filter pore size 0.2 ~).

Furosine was measured by high performance liquid chromatography (HPLC) on an ODS-120A column, 4.6mm x 25cm. the column was equilibrated and eluted with 7mM H_3PO_4. A 5 µl portion of the sample was applied to HPLC. The area under the peak that appeared with a retention time of 4.1 minute under the following condition was measured;
Flow rate: 1ml/min
UV detector wave length: 280nm and 254nm
Detector sensitivity: 0.04 absorbance units of full scale.

A ratio of peak heights of furosine at A-280 nm and A-254 nm (3.9:1) was used for the identification of furosine. The ratio of furosine was expressed as the ratio of the area under the furosine peak to that under the tyrosine peak in the various samples.

RESULTS

Table 1:The mean value of these biochemical parameters of diabetes (Furosine, HbA1c and FBS) were compared with that of control it depicted significantly high level (0.001) of furosine, HbA1c and FBS whereas total protein does not discriminate the difference and shows non-significant value in diabetes as compared to control.

Table 1

Comparison of furosine, HbA1c, FBS and Serum protein of Controls and cases (diabetic type II)

Parameters	Controls (n=100)	Cases (n=100)	
	--	Mean ± SEM	P-Value
Furosine (%)	1.1 ± 0.01	3.5 ± 0.09	0.001
HbA1c (%)	6.4 ± 0.05	10.3 ± 0.12	0.001
FBS (mg/dl)	102 ± 0.63	281 ± 3.88	0.001
Total Serum protein (g/dl)	6.5 ± 0.09	6.4 ± 0.06	0.548

Statistically significant $p<0.05$, Highly significant $p<0.01$

Table 2:

This table shows correlation coefficient (r) in controls. Furosine was correlated positively with HbA1c and FBS with high significance ($p<0.01$). Similarly HbA1c was positively correlated with FBS with high significance ($p<0.01$).

Correlation Coefficient (r) in Cases (diabetic type II)

Correlation	Cases
FurosineVs HbA1c	r = 0.94 *
FurosineVs FBS	r = 0.92 *
HbA1c Vs FBS	r = 0.88 *

* Significant correlation $p<0.01$

DISCUSSION

Diabetes is a clinical syndrome characterized by hyperglycemia due to absolute or relative deficiency of insulin[12]. Glycation is the non-specific binding of sugar to proteins and therefore any protein in the living body may undergo glycation. The extent of glycation of plasma proteins, hemoglobin and those tissue proteins which are readily collectable from the living body such as nail and hair has already been used as a clinical indicator of past control of blood glucose levels.

In this study we found that the furosine level in hair was increased significantly in cases when compared to controls. As we had taken scalp hair 12 cm proximal andaccording to the study of Oimomi M et al (1988)[13]scalp hairs growsat a rate of 1cm/month.This indicates that the patients had

high levels of sugar one year back. Thus it is possible to diagnose the uncontrolled levels of blood sugar in diabetic patients for the past several years with the help of this non-invasio method.

The present study showed that the fasting blood sugar level of cases was highly significant as compared to control; it shows that the subject was diabetic patient. TheHbA1c levels in cases were increased significantly as compared to control. As the study ofGoldstein[14]showed that in hemoglobin the rate of synthesis of glycosylated hemoglobin(HbA1c) is principally a function of the concentration of glucose to which the erythrocytes are exposed. The life span of erythrocytes is 90 to 120 days. This means that the patient had high blood glucose level 3 months ago as the HbA1c indicates Glycation 3 months back. Thus we conclude that the significant high levels of HbA1c are the marker of diagnosis chronic hyperglycemia for the past 3 months.

In our study the correlation between the different diagnostic markers of sugar are also positively correlated with high significance. This shows that the glycation of proteins like hemoglobin and hair protein increases with increase of blood glucose level.

CONCLUSION

We concluded that the furosineand HbA1c levels increase in diabetic patients in comparison to the control while total serum protein was unaffected. We suggest that along with HbA1c, the furosine level should also be included for the determination of blood glucose control as the furosine indicates chronic elevated levels blood glucose furthermore it is a non-invasive method.

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