Pakistan Journal of Pharmacology
Vol.38, No.1, January, 2021, pp.35-38

ORIGINAL ARTICLE

ANALYSIS OF DECREASED LEVEL OF PLASMA TRYPTOPHAN IN DIABETIC PATIENTS

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ABSTRACT

Tryptophan is an essential amino acid found in many protein-based foods and dietary proteins including meats, dairy, fruits, and seeds. High-glycaemic index and -glycaemic load meals also increase the availability of tryptophan. The basic aim of the study is to analyze decreased level of plasma tryptophan in diabetic patients. This study was conducted at DHQ teaching hospital Sargodha during January 2018 to march 2018 with the permission of ethical committee of hospital. The data was collected from 100 diabetic patients of both genders. The data was collected through a questionnaire. A separate interview was done face to face with every patient and then they fill the questionnaire. Demographic data and medical history was collected through questionnaire. The demographic data of selected patients shows that fasting glucose levels were significantly higher ($p<0.01$) in the diabetics compared to the non-diabetic subjects, while the plasma tryptophan levels were significantly reduced ($p<0.01$) in the cases than the controls. The concentration of tryptophan was decreased in diabetic subjects (both men and women) compared to the non-diabetics which was evident by the decreased peak height (value on y-axis) in diabetics compared to the controls. It is concluded that decrease in plasma TRP levels in diabetic patients regardless of their gender and these patients also exhibited a greater incidence of memory dysfunction compared to the controls.

INTRODUCTION

Tryptophan is an essential amino acid found in many protein-based foods and dietary proteins including meats, dairy, fruits, and seeds. High-glycaemic index and -glycaemic load meals also increase the availability of tryptophan. Levels of plasma tryptophan are determined by a balance between dietary intake, and its removal from the plasma as a part of its essential role in protein biosynthesis. Tryptophan is the sole precursor of peripherally and centrally produced serotonin. However, the second most prevalent metabolic pathway of tryptophan after protein synthesis is the synthesis of kynurenine, which accounts for approximately 90% of tryptophan metabolism. Kynurenine is the precursor of kynurenic acid, an antagonist at glutamate ionotropic receptors. There is strong evidence implicating the kynurenines in behavioural and cognitive symptoms of neurological disease, however the relationship between the central effects of tryptophan depletion/ supplementation and the kynurenine pathway is as yet not clear.

Diabetes mellitus (DM) is the most common serious metabolic and endocrine disorder characterised by hyperglycaemia resulting from decreased insulin production/secretion. Diabetes causes a variety of functional and structural disorders in the central nervous system (CNS) and the

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peripheral nervous system (PNS). Reports have shown impairments in cognitive function and two-fold risk of affective disorders, dementia and Alzheimer's disease, in diabetes. Several factors are implicated in the pathogenesis of diabetes-induced learning and memory impairments. Previous studies in animals have shown a decline in brain tryptophan (TRP) levels and 5-hydroxytryptamine (5-HT) levels in animal models of diabetes. The basic aim of the study is to analyze decreased level of plasma tryptophan in diabetic patients.

MATERIALS AND METHODS

This study was conducted at DHQ teaching hospital Sargodha during January 2018 to march 2019 with the permission of ethical committee of hospital. The data was collected from 100 diabetic patients of both genders. The data was collected through a questionnaire. A separate interview was done face to face with every patient and then they fill the questionnaire. Demographic data and medical history was collected through questionnaire.

BIOCHEMICAL ANALYSIS

Plasma tryptophan was estimated by taking 0.03ml plasma sample in Eppendorf. Then 0.6ml of per chlorate was added to it. After that, the Eppendorffs were centrifuged at high speed for 5 minutes. The supernatant was separated for analysis using HPLC-UV method. HPLC-UV determination was carried out according to the standard procedure. A 5-II Shim-Pack ODS (Octadecylsilane) separation column of 4.0mm internal diameter and 150mm length was used. Separation was achieved by a mobile phase containing methanol (14%), octyl sodium sulfate (0.023%) and ethylene diamine tetra acetic acid (EDTA) (0.0035%) in 0.1M phosphate buffer at pH 2.9 at an operating pressure of 2000-3000 pounds per square inch.

STATISTICAL ANALYSIS

Student’s t-test was performed to evaluate the differences in roughness between group P and S. Two-way ANOVA was performed to study the contributions. A chi-square test was used to examine the difference in the distribution of the fracture modes (SPSS 19.0 for Windows, SPSS Inc., USA).

RESULTS

The demographic data of selected patients shows that fasting glucose levels were significantly higher ($p<0.01$) in the diabetics compared to the non-diabetic subjects, while the plasma tryptophan levels were significantly reduced ($p<0.01$) in the cases than the controls.

The concentration of tryptophan was decreased in diabetic subjects (both men and women) compared to the non-diabetics which was evident by the decreased peak height (value on y-axis) in diabetics compared to the controls.

DISCUSSION

Tryptophan depletion might affect memory processes by affecting brain TRP levels, thereby decreasing 5-HT synthesis. The results of the study provide a strong evidence for the association between low plasma TRP levels and memory impairment in diabetic patients as compared to the controls. The frequency of the memory dysfunction was similar in men and women diabetics. Diabetes is associated with several adverse effects on the brain resulting primarily from hyperglycaemia due to decreased insulin release. Moderate impairment of learning and memory has been reported in diabetes.

Several investigators have suggested that brain tryptophan levels vary with the changes in free plasma TRP. On the other hand, other researchers4 have emphasized that brain TRP levels are more sensitive to the changes in total plasma TRP or to the ratio of total plasma TRP to the sum of large neutral amino acids (LNAA) that compete with TRP for entry into the brain.

Table 1: Demographic values of patients

Variable	Whole sample	Normal blood pressure	High blood pressure	Normal blood pressure through medication	*P*-value
Participants (n)	332	166	112	54	
Age (y)	50 (15)	42 (13)	57 (14)	59 (11)	0.000
BMI (kg/m²)	29 (6)	27 (5)	30 (6)	31 (6)	0.000
%FFM	68 (10)	69 (10)	67 (9)	65 (9)	0.015
%FM	32 (10)	31 (10)	33 (9)	35 (9)	0.015
Waist circumference (cm)	97 (13)	92 (13)	101 (14)	100 (12)	0.000
Gender (%)					
Male	38	38	45	24	
Female	62	62	55	76	
Systolic BP (mm Hg)	129 (18)	118 (11)	148 (14)	125 (9)	0.000
Diastolic BP (mm Hg)	80 (11)	74 (8)	89 (11)	77 (7)	0.000

Table 2: level of tryptophan in diabetic and non-diabetic patients

Parameters	Non-diabetic patients (n=100)		Diabetic patients (n=100)		P-value
	Males	Females	Males	Females	
Age (years)	45±4.6	43±4.5	45±4.4	42.4±4.2	-
Weight (kg)	74±3.7	74±3.6	70±3.6	69±3.4	-
Systolic BP (mm Hg)	122.7±3.4	120.2±3.0	136.4±4.6	132.3±4.4	-
Diastolic BP (mm Hg)	76.4±2	70.5±1.8	84.3±2.8	78.2±2.6	-
Fasting plasma glucose (mg/dl)	76.9±5.2	76.9±5.2	240.6±3.9	238.4±3.8	<0.01
Plasma Tryptophan (g/ml)	18.14±6.13	17.14±6.05	10.99±4.8	10.90±3.1	<0.01

Evidence shows that diabetes is responsible for elevation of the plasma levels of LNAA4, which is linked to reduced central tryptophan uptake18 due to which tryptophan transport to brain is decreased, leading to diminished brain serotonin synthesis rate among the diabetics.

CONCLUSION

It is concluded that decrease in plasma TRP levels in diabetic patients regardless of their gender and these patients also exhibited a greater incidence of memory dysfunction compared to the controls. The decrease in plasma TRP levels in the present findings may be attributed to the greater metabolism of TRP by alternative pathways. Evidence shows that activity of liver TRP oxygenase enzyme is increased in diabetes. Indeed, such metabolic alterations in diabetes may ultimately result in decreased synthesis of brain 5-HT in diabetic patients.

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