ORIGINAL ARTICLE

Role of decreased Plasma Tryptophan in memory deficits observed in Type-I diabetes

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Abstract

Objective: To investigate the relationship between plasma tryptophan and the occurrence of memory dysfunctions in male and female type 1 diabetics.

Methods: The case-control study was conducted at two urban healthcare facilities in Karachi from January to June 2009, and comprised 100 diabetic subjects of among whom were 50 men and 50 women. The controls were also similar in number and gender. A questionnaire was used to evaluate the memory impairment in the subjects. Plasma tryptophan was determined by high performance liquid chromatography with ultra-violet method. Students' t test was used to analyse tryptophan data.

Results: There was considerable memory impairment in the cases (n=40) compared to the controls (n=5). Results also showed a significant ($p<0.01$) decrease in plasma tryptophan levels in both male and female diabetic patients.

Conclusions: Diabetic subjects exhibited occurrence of memory impairment with concomitant decline in plasma tryptophan levels. The findings indicate that decreased brain uptake of tryptophan and lowered brain 5-hydroxytryptamine levels may be responsible for the memory deficits seen in diabetics.

Keywords: Tryptophan, 5-HT, Diabetes, Memory Impairment. (JPMA 63: 65; 2013)

Introduction

Diabetes mellitus (DM) is the most common serious metabolic and endocrine disorder characterised by hyperglycaemia resulting from decreased insulin production/secretion.[1,2] Diabetes causes a variety of functional and structural disorders in the central nervous system (CNS) and the 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.[3] Several factors are implicated in the pathogenesis of diabetes-induced learning and memory impairments.[3] Previous studies in animals have shown a decline in brain tryptophan (TRP) levels and 5-hydroxytryptamine (5-HT) levels in animal models of diabetes.[4,5]

Serotonin has been implicated in learning and memory functions.[6] Previous studies have suggested that administration of TRP, the dietary precursor of 5-HT, increases brain 5-HT levels and improves memory functions.[7] Decreased 5-HT levels, on the other hand, have been associated with impaired memory functions.[8] Reports have shown that 5-HT synthesis and release is directly related to tryptophan availability.[4] Tryptophan depletion, a challenge tool for 5-HT, has been shown to induce memory deficits in humans,[9] but on affective behaviour, its effect is modest.[10] Alteration in metabolism and neurotransmission of brain 5-HT may be related to neuropsychiatric disorders observed in diabetes.[5] The aim of the present study was to relate the plasma TRP levels with the occurrence of memory dysfunction commonly observed in diabetic subjects.

Patients and Methods

The case-control study was conducted from January to June 2009 in Karachi, Pakistan, and involved 100 patients diagnosed with diabetes type I from two private-sector hospitals and 100 non diabetic healthy individuals. Both the groups had 50 men and 50 women subjects age ranging between 35 and 55 years. The study was done in accordance wit the ethical recommendations and practices of the two hospitals.

In separate interviews, the subjects filled up a questionnaire regarding their medical history. Patients with type 2 diabetes mellitus and hypertension were excluded from the study.

The non-diabetic controls were of the same age as the diabetics with normal glucose tolerance test and they were not on any medical treatment during the course of the study. Fasting blood samples from all the participants were collected from the anticubital vein. Plasma was separated and used for the determination of the glucose and plasma TRP levels. Plasma glucose was determined by O-Toulidine method and plasma TRP levels were measured by high performance liquid chromatography

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with ultra-violet (HPLC-UV) method. Samples for analysis were stored at -70°C.

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 5minutes. The supernatant was separated for analysis using HPLC-UV method. HPLC-UV determination was carried out according to the standard procedure.[11] 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 ethylenediaminetetraacetic acid (EDTA) (0.0035%) in 0.1 M phosphate buffer at pH 2.9 at an operating pressure of 2000-3000 pounds per square inch on Schimadzu LEC 6A detector at an operating potential 1.0 V for TRP.

Tryptophan data analysis was done using student t- test. Values of $p<0.05$ were considered significant.

Results

The general characteristics as well as plasma glucose and

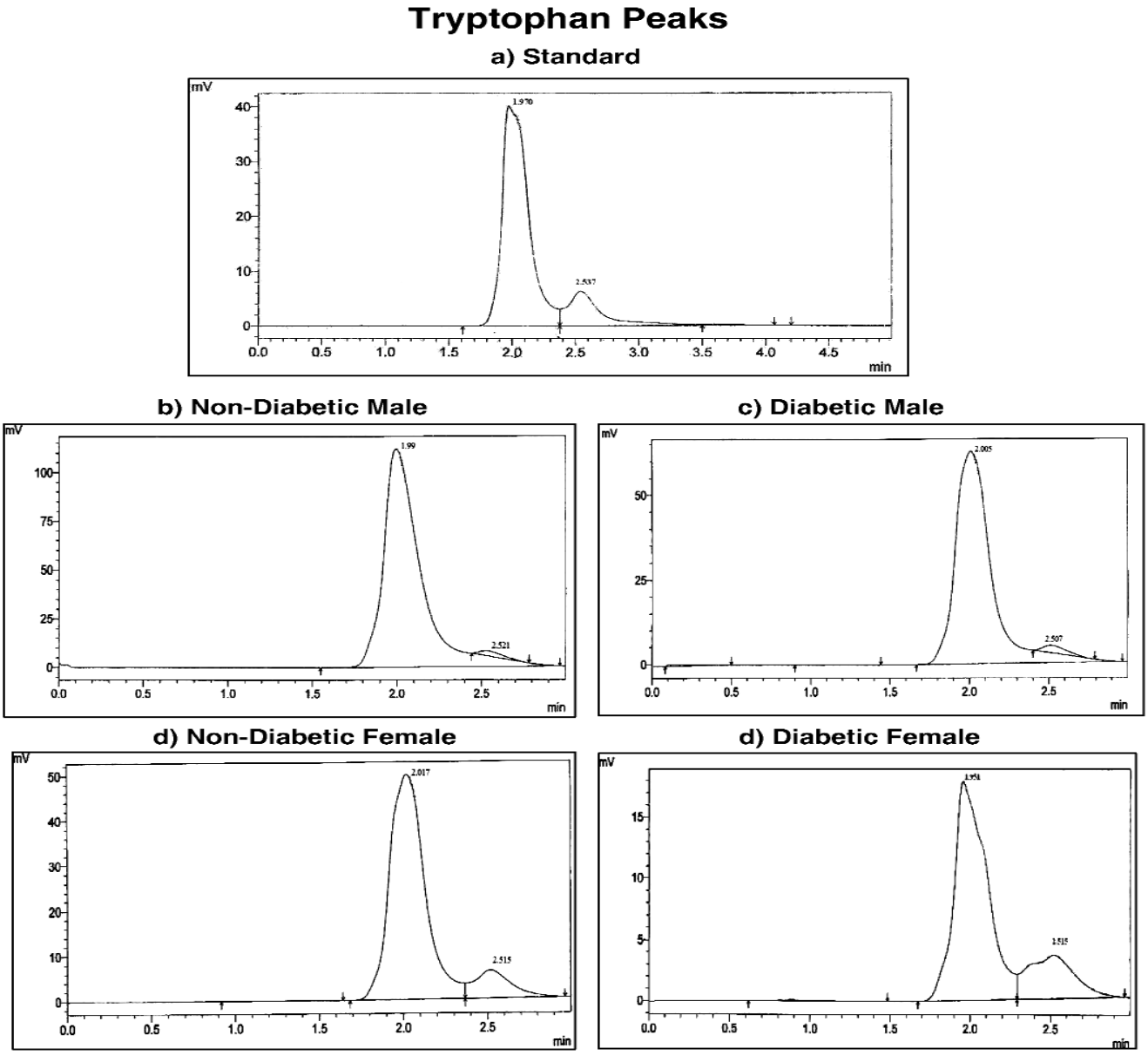

Figure-2: Trace of HPLC-UV chromatograms showing separation of tryptophan; a) in standard (retention time 1.970); b) in non-diabetic males (retention time 1.99); c) in diabetic males (retention time 2.005); d) in non-diabetic females (retention time 2.017); e) in diabetic females (retention time 1.951).

Table: Comparison of characteristics of the cases and the controls.

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

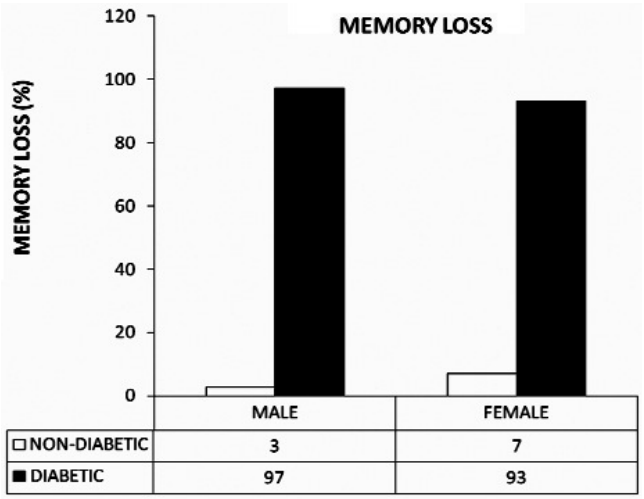

Figure-1: The occurrence of the memory dysfunction in terms of percentage in diabetic and non-diabetic subjects.

tryptophan levels of the cases and the controls were compared (Table). 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.

Memory impairment was greater among the diabetics (n=40; 40%) than the healthy subjects (n=5; 5%) (Figure-1).

HPLC-UV showed that 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 (Figure-2). From this peak height, concentration of tryptophan was calculated in μg/ml of plasma.

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.[12] Moderate impairment of learning and memory has been reported in diabetes.[2,13]

The current study demonstrated a 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. Several investigators[14-16] have suggested that brain tryptophan levels vary with the changes in free plasma TRP. On the other hand, other researchers[4] have emphasised 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. Evidence shows that diabetes is responsible for elevation of the plasma levels of LNAA[4,17] which is linked to reduced central tryptophan uptake[18] due to which tryptophan transport to brain is decreased, leading to diminished brain serotonin synthesis rate among the diabetics.

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.[19,20] Indeed, such metabolic alterations in diabetes may ultimately result in decreased synthesis of brain 5-HT in diabetic patients. Concentration of TRP in blood is known to be an important determinant of brain 5-HT. Increase in brain 5-HT depends on the increase in brain tryptophan as the enzyme tryptophan hydroxylase is half saturated with its substrate.[21,4] Thus, increased concentration of brain TRP increases brain 5-HT and reduces levels of plasma TRP, leading to decreased brain TRP and decreased brain 5-HT concentrations.[22] Reports have shown that tryptophan uptake by brain was decreased in diabetic condition,[23] leading to reduction in brain tryptophan levels due to which synthesis and turnover of 5-HT in brain was also

decreased.[22]

Evidence shows that brain TRP and 5-HT have a significant role in learning and memory.[6,20] Alterations in brain 5-HT levels can contribute to behavioural differences in mice and psychiatric disorders in humans.[24] Previously, it has been reported that increasing brain 5-HT by administration of TRP enhances memory function,[6] while decreased brain 5-HT has also been shown to impair memory.[25] The present study suggests that decreased plasma TRP levels in diabetes may be responsible for the impaired memory functions observed in diabetic patients.

Conclusion

The findings suggest that the altered brain 5-HT metabolism may be the reason behind memory deficits commonly observed in diabetics. Larger studies are needed to reveal the potential of diabetes to cause memory impairment due to decreased serotonin levels in the brain, and to explore the possible cause, cure and management of diabetes to control the effects of memory loss in the diabetics.

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