Research article

Serum tryptophan, insulin, and serotonin in type 2 diabetic patients: A pilot study

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ABSTRACT

Introduction and Aim: T2DM being a most prevalent metabolic disorder globally and is mainly triggered by two important factors: decreased insulin secretion by pancreatic β-cells and the defective insulin-sensitivity of tissues. Recent studies found that other factors influence insulin release are amino acids, fatty acids, incretins, certain neurotransmitters, and some of the pituitary hormones. Higher levels of peripheral serotonin in diabetic people have drawn attention to the involvement of metabolic homeostasis and cellular sensors. Thus, the aim of this study is to screen the association of tryptophan, serotonin, and insulin in T2DM.

Materials and Methods: Serum samples from T2DM patients (n=80) were divided into 2 groups depending on HbA1C levels having good to moderate (6.5-8) and poor (>8) glycemic control to study the levels of tryptophan, serotonin, and insulin.

Results: A boarderline statistically significant increase was observed in Serotonin levels in group 2 when compared with group 1. Statistical analysis showed a negative correlation between insulin and serotonin in group 1, whereas in group 2 a negative correlation has observed between HbA1C and insulin.

Conclusion: The study report shows an increase in serum serotonin levels with increase in insulin levels and hyperglycemia.

Keywords: T2DM; serotonin; insulin; HbA$_{1C}$; tryptophan.

INTRODUCTION

Type 2 diabetes (T2DM) being a most prevalent metabolic disorder globally and is mainly triggered by two important factors: decreased insulin secretion by pancreatic β-cells and the defective insulin-sensitivity of tissues (1). David *et al.,* have reported that in 2011, there were 366 million people with diabetes, which may rise to 552 million by 2030 (2). T2DM is also causative for the increased risk of developing cardiovascular disease, coronary heart disease, diabetic neuropathy, nephropathy (3) and a variety of cancers. Recent metabolomics screens have observed tryptophan metabolites as potential biological mediators in the development of T2DM (4-6). Tryptophan is an essential aromatic amino acid (7), the body uses it for a wide range of physiological processes, including the control of immunological responses, mood and behavior, growth, and diet (8). Serotonin is one of the biologically important products formed from tryptophan (Trp). Trp is hydroxylated to produce 5-hydroxytryptophan as the first step in the metabolic cascade (5-HTP, oxitriptan). Tryptophan hydroxylase (TPH), which has two isoenzymes, catalyzes this process. TPH1 is active in peripheral tissues (such as the skin and gastrointestinal tract), whereas TPH2 is active in neuronal cell types. The carboxyl group is then removed by aromatic amino acid decarboxylase (AAAD), resulting in the formation of 5-hydroxytryptamine (5-HT, serotonin; 9). Serotonin plays multiple roles during energy homeostasis. Being a neurotransmitter, in the brain it regulates anxiety and appetite-related behaviors (10). Recently, the higher levels of peripheral serotonin in diabetic people have drawn attention due to its involvement in metabolic homeostasis and cellular sensors (11).

After preloading with the 5-HT precursor 5-hydroxytryptophan, 5-HT has long been known to co-localize with insulin in pancreatic -cells, where it has been linked to the control of blood glucose levels. Islet 5-HT raises the β-cells' sensitivity to glucose through the 5-HT3 receptor, which in turn increases the amount of islet glucose-stimulated insulin secreted overall. Given that β-cells produce 5-HT during the perinatal period and pregnancy, which are two physiological conditions of -cell proliferation; 5-HT is believed to be a key regulator of -cell proliferation and insulin secretion (12).

Studies have found that numerous metabolites are associated with insulin resistance and T2DM (9). Some of the metabolic profiles can help to assess the future development of T2DM well before (10 to 15 years) the onset of the disease. Some amino acids, particularly L-glutamine and L-leucine alone or in combination, or L-arginine alone, can increase intracellular Ca^{2+} levels to promote the release of

insulin, through / by various processes. Recent research has shown that neurotransmitters and their receptors produced in β-cells have some impact on insulin release. Evidence suggests that the signals from serotonergic and enteric nervous systems also shows regulatory actions on release of insulin (13). Ninety-five percent of serotonin is synthesized in the periphery, specifically in the intestine, and its synthesis is also influenced by intestinal flora. It induces inflammation in the periphery, inhibits brown adipose tissue function, and increases during obesity (14,15). Furthermore, its effect on homeostasis of blood glucose, gluconeogenesis, and the hepatic free fatty acid mobilization (16) is well documented. Hence, serotonin may be involved directly in the development of metabolic disorders. With this knowledge we aimed to study the association of tryptophan, serotonin, and insulin in T2DM.

MATERIALS AND METHODS

This is an observational pilot study that was conducted for a period of five months after approval by the Institution Scientific Committee. The study population consisted of 80 known type 2 diabetic patients. These patients further divided into 2 groups based on their HbA1C levels as fair to good glycemic control (HbA1C =6.5-8.0) and poor glycemic control (HbA1C >8). The patients included in the study did not have Known liver disorders, pancreatitis, and malignancies and those who are under Insulin therapy, antidepressant therapy is excluded. Leftover samples of T2DM patients collected from Central Clinical Lab (KMCHAC) and stored at -20°C for biochemical analysis. Parameters that are estimated include Insulin, serotonin and Tryptophan.

HbA1C estimated by HPLC method. Insulin is estimated using 'The Calbiotech Inc.,' Insulin ELISA Kit based on sandwich ELISA principle. Serotonin is estimated using Elabscience® ST/5-HT (Serotonin/5-Hydroxytryptamine) ELISA Kit based on the competitive ELISA principle. Tryptophan levels were measured by HPLC.

Statistical analysis

Data was analyzed using EZR statistical software, version 1.54. (Developed by Jichi Medical University, Saitama Medical Center, Tokyo, Japan). Normal data are expressed as Mean ± Standard deviation. Non-normal data are expressed as median with interquartile range taking 95% confidence interval. For normal data independent sample-t-test (two sample t tests were used). For non-normal data Mann-Whitney U Test was done. Spearman's rank Correlation was carried out to find co-relation coefficient.

RESULTS

A borderline significant increase in serotonin levels in Group 2 was observed.

Table 1: Levels of HbA1C, serotonin and insulin

Parameters	Group 1 (n=40)	Group 2 (n=40)	p value
HbA$_{1C}$ (%)	7.12 ± 0.45	9.92 ± 1.56	<0.001*
Serotonin (ng/ml)	24.07 (20.87, 30.61)	35.86 (23.41,113.87)	<0.049*
Insulin (µIU/ml)	6.90 (4.95, 23.52)	17.46 (6.32, 28.31)	0.227
Tryptophan (µMol/L)	42 (36, 53)	47.5 (36, 61.75)	0.231

Group 1= Good to moderate glycemic control.
Group 2= poor glycemic control.

Table 2: Correlation between parameters in Group1

Parameters	Spearman's Correlation Coefficient (r value)
HbA1C-Insulin	0.02
HbA1C-Serotonin	0.134
Insulin-Serotonin	-0.179
Tryptophan - HbA1C	-0.293
Tryptophan -Serotonin	-0.183
Tryptophan - Insulin	0.222

No significant correlation between the any of the parameters in Group 1. However, there was a negative correlation between insulin and serotonin, tryptophan and insulin and tryptophan and serotonin

Table 3: Correlation between parameters in Group 2

Parameters	Spearman's Correlation Coefficient (r value)
HbA1C-Insulin	-0.202
HbA1C-Serotonin	0.175
Insulin-Serotonin	0.015
Tryptophan - HbA1C	-0.016
Tryptophan -Serotonin	-0.097
Tryptophan - Insulin	-0.09

No significant correlation between any of the parameters in group 2. However, there is a negative correlation between HbA1C and insulin, tryptophan and HbA1C and tryptophan and serotonin & tryptophan and insulin.

DISCUSSION

Before discussing the results, we should consider this work as a pilot for future studies under more controlled conditions or parameters. T2DM is characterized by disturbances in metabolic processes and hyperglycemia. Diabetes can be caused by both environmental and biological factors. The global incidence of T2DM has increased significantly because of food habits and lifestyle. This study is focused on correlation of insulin, serotonin, and tryptophan with T2DM having good to moderate and poor glycemic control. T2DM starts with peripheral insulin resistance. Pancreatic beta-cells respond by producing more insulin and proliferating more frequently to counteract this resistance (17). It is

widely recognized that in type 2 diabetic patients, b-cell function worsens over time, making it difficult to achieve adequate glycemic control without the use of insulin (18). It has been shown that dysregulation of the kynurenine pathway may be involved in the pathogenesis of DM.

According to recent findings, increasing serotonin levels should inhibit glucagon release, a hormone with significant hyperglycemic qualities (19). In the periphery, through a variety of receptors and intricate signaling processes, serotonin plays a significant role in energy metabolism due its production at multiple cellular sites and its efficiency to act as an auto, para, and endocrine aspect. Specifically, gut derived serotonin act on different tissues to regulate glucose and lipid metabolism (20,21). Serotonin from pancreas enhances insulin secretion and β cell proliferation during pregnancy to meet the enhanced requirement of insulin (17). It promotes white adipose tissue lipolysis, stimulates hepatic gluconeogenesis and inhibits glucose uptake. In brown adipose tissues serotonin deprives thermogenesis and glucose uptake. Additionally, it enhances glycolysis in muscles. It is also noted that serotonin modifies the inflammatory processes through macrophages (22).

In the present study, we have observed an increase in the serum serotonin levels in the group which includes T2DM with poor glycemic control in comparison with good to moderate control as per their HbA1C levels. A similar observation was reported by Takada *et al.,* (23), where serotonin level always high in T2DM when compared to normal. Recent investigations on animal and cell types have revealed a variety of peripheral serotonin activities using target-specific receptors or rate-limiting enzymes of serotonin production. These effects are triggered by targeting the peripheral serotonin synthesis components and signaling shows promising therapeutic strategies for treating diabetes. Antidepressants like some selective 5-HT reuptake inhibitors (SSRIs), which raise 5-HT levels in the synaptic cleft and enhance insulin sensitivity and glucose metabolism, are known to influence 5-HT metabolism and affect glucose homeostasis (24). Al-Zoairy *et al.*, have reported that cellular uptake of 5-HT is dose and time dependent. It also enhances uptake of glucose from skeletal muscle, increasing glycogen content in the absence of insulin. All these processes remained augmented in the presence of insulin (25).

CONCLUSION

Present study results show an increase in serum serotonin levels and are increased with decreased glucose tolerance/high HbA1C. This could be an adaptation to increase the release of insulin in a positive way or an effect of enhanced inflammation, ROS production and other associated metabolic alterations concomitant with poor glycemic control.

Though the results are not significant, the study shows that serotonin and tryptophan are involved in the pathogenesis of T2DM and modulation of glycemic status. Hence, a detailed and more focused with careful selection of study subjects will help to reveal the mechanisms associated with an increase in serotonin levels in subjects with altered glycemic control.

CONFLICT OF INTEREST

Authors declare no conflicts of interest.

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