WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 8, 729-736. Research Article ISSN 2277– 7105

A HOSPITAL BASED STUDY ON CORRELATION BETWEEN HYPERGLYCEMIA, GLYCATED HEMOGLOBIN, LIPID AND OXIDATIVE STRESS VARIABLES IN TYPE 2 DIABETES MELLITUS SUBJECTS: A CROSS SECTIONAL ANALYSIS.

Zephy Doddigarla[1], Iqbal Parwez[2] and Jamal Ahmad*[3]

[1,3]Rajiv Gandhi Centre for Diabetes and Endocrinology, A.M.U., Aligarh.

[2]Department of Zoology, A.M.U., Aligarh.

Article Received on
25 May 2016,

Revised on 16 June 2016,
Accepted on 07 July 2016

DOI: 10.20959/wjpr20168-6701

***Corresponding Author**
Prof. Jamal Ahmad
Rajiv Gandhi Centre for Diabetes and Endocrinology, A.M.U., Aligarh.

ABSTRACT

OBJECTIVE: The present study was designed to test whether melatonin has an effect on glucose, lipids, and oxidative stress variables in type 2 diabetes mellitus (T2DM) subjects. **DESIGN & METHODS:** Fifty T2DM patients and fifty healthy individuals were included in the study. T2DM diagnosis was made according to norms laid by American Diabetes Association. Fasting blood sugar (FBS) and Lipid profile were quantified by commercial kits. Glycated hemoglobin (HbA1c) was estimated by HPLC method. Serum malondialdehyde (MDA), total antioxidant capacity, catalase, and nitric oxide were determined. Reduced glutathione was also determined by the method of Ellman's. The oxidative stress variables were measured with micro-volume spectrophotometer. Melatonin was estimated using the radioimmunoassay kit. **RESULTS:** The authors observed low melatonin and glutathione levels and increased FBS, total cholesterol, triacylglycerols, low density lipoproteins, HbA1c, and MDA levels in T2DM group compared with healthy controls. Further in the patient group, a positive correlation was observed between MDA and FBS (R^2=0.2681), HbA1C and FBS (R^2=0.381), HbA1C and MDA (R^2=0.348) and the negative correlation between HbA1c and melatonin (R^2=-0.363). In the control group, we found positive correlation between glutathione and melatonin (R^2=0.569), and negative correlation between HbA1c and melatonin (R^2=-0.329), MDA and melatonin (R^2=-0.297) respectively. **CONCLUSION:** We conclude that lipid peroxidation increases in patients with T2DM, is due to melatonin deficiency, oxidative stress and T2DM. Antioxidant defense system in normal aging is able to compensate for the

peroxidation products. However, this compensation mechanism may become insufficient due to the decreased melatonin secretion in the aging subjects who are prone to T2DM.

KEYWORDS: Type 2 Diabetes Mellitus, Oxidative stress, Aging, Melatonin, Malondialdehyde, Glutathione.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is an endocrine disorder of pancreas characterized by deficient action of insulin on insulin sensitive tissues and/or relative lack of insulin which leads to hyperglycemia. Hyperglycemia subsequently induces dyslipidemia and reactive oxygen species (ROS) that implicate oxidative stress and thus, cause selective activation of pathways including polyol, protein kinase C, hexosamine, and advanced glycation end products which progress pathogenesis of diabetes.[1,2] Many hypotheses exist in the development of T2DM including genetic, lifestyle, obesity, and deficiency of melatonin. Interestingly, T2DM is considered as an aging disorder[2] and melatonin deficiency occurs due to decrease in pineal gland size as the age advances.

Melatonin is useful in insulin action based on the finding that pinealectomy induces insulin resistance.[3] Genome-wide studies in French and Han Chinese subjects, identified rs1387153 variant near MTNR1B (which encode the melatonin receptor 2 (MT2) as a modulator of fasting plasma glucose.[4,5] Further in another study on European population, the rs1387153T allele is associated with increased fasting plasma glucose and risk of developing T2DM over a 9-year period.[6] On the other side, some studies after supplementation of melatonin observed improvement in fasting blood sugar, total cholesterol (TC), and malondialdehyde (MDA, which is an index of lipid peroxidation) in metabolic syndrome individuals, at baseline these variables were altered.[7] Further, melatonin is profoundly known as antioxidant hormone for its action at reducing oxidative stress.[8,9] Nevertheless, these findings imply that melatonin deficiency leads to hyperglycemia, dyslipidemia, and oxidative stress.

The hypothesis of the present study is to test whether melatonin has an effect on glucose, lipids, and oxidative stress variables in T2DM subjects.

MATERIAL AND METHODS

The present hospital based study was conducted at Rajiv Gandhi Centre for Diabetes & Endocrinology, a tertiary care of Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, India. The study protocol was approved and cleared by institution ethics review board for human studies and patients have signed an informed consent. Since there is no computerized data available; it is a cumbersome task to collect the data manually, therefore, the sample size has been confined to fifty T2DM patients. T2DM individuals who were on hypoglycemic drugs without complications and not taking supplementations were included in the group. The exclusion criteria were type 1 diabetes individuals, less than five years of known duration of T2DM, and with complications. The diagnosis of T2DM was made according to the norms laid by American Diabetes Association. The inclusion criteria for healthy controls (n=50), who were non-diabetic, not taking supplementations, and having no other complications. All the ethical norms were carried out while collecting the blood. Fasting venous blood (5ml) was drawn into grey (for plasma) and red (for Serum) tops vaccutainers for estimation of study variables under all aseptic conditions. Serum/Plasma was separated by centrifugation of the blood at 3000 rpm for 20 minutes. Serum samples were stored in aliquots at -80˚ C until assayed.

Analytical methods

Plasma glucose was estimated by GOD-POD Kit method. Glycated hemoglobin (HbA1c) was estimated by HPLC method by using HbA1c variant instrument. TC, TAGs and HDL cholesterol were quantified by kits, which were obtained from Avantor Performance Materials India Limited, Dehradun, Uttarakhand, India. The fasting glucose and lipid variables were measured with Lablife chem-master semi-autoanalyser. Thiobarbituric acid reacting substances level was measured as an index of MDA production and so lipid peroxidation, was achieved by the method of Ohkawa et al., 1979.[10] Total antioxidant capacity (TAC) was estimated by the Pohanka et al method.[11] Catalase (CAT) was performed by the method of.[12] Nitric Oxide (NO) in serum was determined indirectly according to the method of Griess L. et al., 1982.[13] Reduced glutathione (GSH) was determined by the method of Ellman's.[14] The oxidative stress variables were measured with MySpec micro-volume spectrophotometer. Melatonin was estimated using the radioimmunoassay kit obtained from LDN Labor Diagnostika Nord GmbH & Co. KG with the instrument I'acn videogamma/Rack, PC-RIA.MAS STRATEC.

All chemical reagents were of analytical grade purchased from Merck and Sigma agents. The

study variables methods for standards and samples were performed in duplicate. The intra-assay and inter-assay coefficient variations for study methods were between 5%- 10%.

STATISTICAL ANALYSIS

Results are given as mean±SD. The present study allowed us to estimate the SD and the difference between the means. The statistical differences between the groups was determined by student 't' test. Pearson correlation was also done to know the association between two variables. Linear regression analysis was used as appropriate. A p value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

We observed low melatonin (t=10.165,d=98) and GSH (t=3.829,d=98) levels and increased FBS (t=6.955,d=98), TC (t=5.043,d=98), TAGs (t=2.419,d=98), LDL (t=3.456,d=98), HbA1C (t=10.931,d=98), and MDA (t=14.412,d=98) levels in T2DM group compared with healthy controls (Table 1). Further, we observed positive correlation between FBS and MDA (R^2=0.2681,P=0.049), HbA1C and FBS (R^2=0.381,P=0.006), HbA1C and MDA (R^2=0.348,P=0.013) in T2DM group. Moreover, we also observed a negative correlation between HbA1C and melatonin (R^2=-0.363,P=0.009). These findings suggest that increase in ROS generation due to autoxidation of glucose and in addition, dyslipidemia added an insult to the antioxidant status, although the authors did not observe correlation between oxidative stress variables of the study and the dyslipidemia values. However, significant difference was observed in TC, TAGs, and LDL values between T2DM subjects and healthy controls (Table 1). The lipid peroxidation (MDA, which is an indicator of ROS attack on lipids) in T2DM may be caused by hyperglycemia and dyslipidemia resulted ROS. Previous findings also demonstrated hyperglycemia and dyslipidemia in T2DM.[15] We suspect that increased lipid peroxidation and decreased melatonin and GSH levels in patients with T2DM may be responsible for the risk relation between oxidative stress and T2DM and are important factors in the development of oxidative stress. This study also shows that increase in oxidative stress enhances the non-enzymatic glycation of proteins including hemoglobin. A study has shown that hyperglycemic patients have lower GSH in T2DM.[16] Melatonin was observed to be lower in the present study, probably due to clearing up of ROS. Nevertheless, it seems that MDA increase alone is not enough for the development of oxidative stress in T2DM. This could imply that the administration of compounds that could raise the levels melatonin and GSH may be therapeutically beneficial.

Table 1. Findings in patients with T2DM and healthy control group

Variable	T2DM group (n=50)	Control group (n=50)	P- value
FBS (mg/dL)	132.5±44.9	86.8±11.3	S
TC (mg/dL)	167.5±27.7	145.6±12.1	S
TAGs (mg/dL)	158.9±55.3	136.5±21.3	S
HDLc (mg/dL)	43.7±4.6	45.7±3.4	NS
LDL (mg/dL)	89.4±7.1	66.2±4.2	S
HbA1c (%)	7.3±1.4	4.8±0.6	S
MDA (nmol/mL)	5±0.9	2.3±0.6	S
Melatonin (pg/mL)	14.4±4.2	35.2±12.8	S
TAC (µmol/dL)	2.6±0.9	4.2±0.4	NS
CAT (U/mL)	2.1±0.7	2.4±0.3	NS
GSH (µmol/L)	18±5.3	24.9±6.1	S
NO (µmol/mL)	13.6±2.3	11.1±2.9	NS

Note: FBS- fasting blood sugar, TC-total cholesterol, TAGs-triacylglycerols, HDLC-high density lipoprotein cholesterol, LDL-low density lipoproteins, HbA1c-glycated hemoglobin, MDA-malondialdehyde, TAC-total antioxidant capacity, SOD-superoxide dismutase, CAT-catalase, GSH-glutathione, NO-nitric oxide; S-significant (<0.05), NS-not significant (>0.05)

The results of the present study show that one of the reasons for oxidative stress in T2DM group could be due to melatonin deficiency. Hyperglycemia increases the non-enzymatic glycation of proteins[17] that may have impaired the production of antioxidants at the molecular level in the present study. Melatonin on the other hand, due to its lower levels in the patient group could not synthesize the genes of antioxidant. Melatonin indirectly or directly, increases the antioxidant enzymes by enhancing the synthesis of antioxidant gens by activating nrf-2 gene and decrease ROS.[18] This means that deficiency in melatonin decrease seems to be related to oxidative stress. However, low melatonin levels in T2DM group and statistically differences between healthy controls and patient group in our study could explain not only by a decrease in melatonin due to hyperglycemia and ROS, but also by a decrease in melatonin due to T2DM, which is probably higher as the age advances above 40 years.

It is interesting that a correlation between melatonin and HbA1c was negative in controls and T2DM patients respectively. At first it seems contradictory, but possible explanation could be that in control group melatonin is higher than in the T2DM group. Therefore, it has been well known that melatonin enhances the production of antioxidant genes.

Hyperglycemia and ROS can cause low GSH levels. Since T2DM patients in the present

study had low GSH levels, our study thoroughly supports this view. GSH plays a very important role in free radical reduction. Several studies have indicated a disruption of GSH levels in diabetic patients.[7,8,19] As GSH maintains intracellular redox potential, any change in redox couple molecules may hamper the GSH levels. For example, NADPH/NADP ratio that is altered due to increased functioning of aldose reductase enzyme in sorbitol pathway, which is increased in diabetic patients.[20] Melatonin is a good provider in maintaining redox coupling of GSH.[19] The presence of significant lower melatonin and GSH levels in patient group may be seen as a remarkable determinant of oxidative stress.

Positive correlation between GSH and melatonin observed in the control group points to the balance between oxidant and antioxidant system, and absence of such a correlation in T2DM group, which is due to hyperglycemia, dyslipidemia and ROS is the result of oxidative stress. Indeed, several reports have indicated oxidative stress in T2DM.[6,18]

We conclude that lipid peroxidation increases in patients with T2DM, is due to melatonin deficiency, oxidative stress and T2DM. Antioxidant defense system in normal aging is able to compensate for the peroxidation products. However, this compensation mechanism may become insufficient due to the decreased melatonin secretion in aged individuals who are prone to T2DM.

REFERENCES

1. Rains JL, Sushil KJ. Oxidative Stress, Insulin Signaling, and Diabetes. Free Rad Bio Med, 2011; 50(5): 567-5.
2. Defronzo RA. From The Triumvirate to the Ominous Octet: A New Paradigm for the Treatment of Type 2 Diabetes Mellitus. Diabetes, 2009; 58(4): 773-5.
3. Peschke E, Bahr I, Muhlbauer E. Experimental and clinical aspects of melatonin and clock genes in diabetes. J Pineal Res., 2015; 59: 1-23.
4. Lyssenko V, Cecilia LFN, Michael RE, et al. Common Variant in MTNR1B Associated With Increased Risk of Type 2 Diabetes and Impaired Early Insulin Secretion. Nature Gen, 2009; 41(1): 82-8.
5. Rönn T, Wen J, Yang Z, et al. A Common Variant in MTNR1B, Encoding Melatonin Receptor 1B, is Associated with Type 2 Diabetes and Fasting Plasma Glucose in Han Chinese Individuals. Diabetologia, 2009; 52(5): 830-3.

6. Bouatia-Naji N, Amélie B, Christine C-P, et al. A Variant Near MTNR1B Is Associated With Increased Fasting Plasma Glucose Levels And Type 2 Diabetes Risk. Nature Gen, 2009; 41(1): 89-94.
7. Kozirog M, Adam RP, Piotr D, et al. Melatonin Treatment Improves Blood Pressure, Lipid Profile, And Parameters Of Oxidative Stress In Patients With Metabolic Syndrome. J. Pineal Res., 2011; 50(3): 261-6.
8. Zhang HM, Zhang Y. Melatonin: a well documented antioxidant with conditional pro-oxidant actions. J Pineal Res., 2014; 57: 131-146
9. Manchester LC, Coto-Montes A, Boga JA et al. Melatonin: an ancient molecule that makes oxygen metabolically tolerable. J Pineal Res., 2015; 59: 403-419.
10. Ohkawa H, Nobuko O, Yagi K. Reaction of Linoleic Acid Hydroperoxide with Thiobarbituric Acid. J Lip Res., 1978; 19(8): 1053-7.
11. Pohanka M, Hana B, Jakub S, et al. Ferric Reducing Antioxidant Power And Square Wave Voltammetry For Assay Of Low Molecular Weight Antioxidants In Blood Plasma: Performance And Comparison Of Methods. Sensors, 2009; 9(11): 9094-3.
12. Luck H. Catalase, Bergmeyer HU (Ed.). Methods of Enzymatic Analysis. Academic Press; New York, 1971; 3: 885-893.
13. Sun J, Zhang X, Broderick M, et al. Measurement of Nitric Oxide Production In Biological Systems By Using Griess Reaction Aassay. Sensors, 2003; 3(8): 276-4.
14. Ellman MA. Spectrophotometric Method for Determination Of Reduced Glutathione In Tissues. Analyt. Biochem, 1959; 74: 214-6.
15. Ståhlman M, Björn F, Martin A, et al. Dyslipidemia, But Not Hyperglycemia and Insulin Resistance, Is Associated With Marked Alterations in The HDL Lipidome in Type 2 Diabetic Subjects In The DIWA Cohort: Impact On small HDL Particles. Mol Cel Bio Lipids, 2013; 1831(11): 1609-7.
16. Chen H, Ming Y, Ming L, et al. Polymorphic Variations In Manganese Superoxide Dismutase (Mnsod), Glutathione Peroxidase-1 (GPX1), And Catalase (CAT) Contribute To Elevated Plasma Triglyceride Levels In Chinese Patients With Type 2 Diabetes Or Diabetic Cardiovascular Disease. Mol Cel Bio., 2012; 363(1-2): 85-1.
17. Arena S, Anna MS, Giovanni R, et al. Non-Enzymatic Glycation And Glycoxidation Protein Products In Foods And Diseases: An Interconnected, Complex Scenario Fully Open To Innovative Proteomic Studies. Mass Spect Rev., 2014; 33(1): 49-7.

18. Kilic U, ErtugruL K, Zeynep T, et al. Melatonin Suppresses Cisplatin-Induced Nephrotoxicity via Activation Of Nrf-2/HO-1 Pathway. Nutr Metab (Lond), 2013; 10(7): 1-8.
19. Loven D, Schedl H, Wilson H, et al. Effect of Insulin and Oral Glutathione on Glutathione Levels And Superoxide Dismutase Activities In Organs Of Rats With Streptozotocin-Induced Diabetes Rats. Diabetes, 1986; 35: 503-7.
20. Eklow L, Moldeus P, Orrenius S. Oxidation of Glutathione during Hydroxide Metabolism: A Study Using Isolated Hepatocyte and The Glutathione Reductase Inhibitor 1, 3-bis (2-Chloroethyl)-1-Nitrosourea. Eur J Biochem, 1984; 138: 459-3.