

Right Dorsolateral Frontal Lobe N-Acetyl Aspartate and Myoinositol Concentration Estimation in Type 2 Diabetes with Magnetic Resonance Spectroscopy

RAJANI SANTHAKUMARI NAGOTHU¹, YOGANANDA REDDY INDLA², ARCHANA RAJAGOPALAN³, RAVI VARMA⁴

ABSTRACT

Introduction: Chronic hyperglycaemia in type 2 diabetes, effects the central nervous system by altering the concentrations of brain metabolites like N-acetyl aspartate (NAA) and myoinositol (ml), which are indicators of neuronal integrity and glial cell damage respectively. Dorsolateral frontal lobe is associated with aspects of cognition especially right frontal lobe is involved in episodic memory retrieval, ninety percent of the diabetic cases are type 2 in nature globally and yoga is very effective in stabilizing the brain metabolites by bringing the blood glucose levels to near or within the physiological range in type 2 diabetes.

Aim and Objectives: The aim of the study was to observe the effects of yogasana and pranayama on glycosilated haemoglobin (HbA1c) levels and right dorsolateral frontal cortical NAA and ml concentration in type 2 diabetic subjects.

Materials and Methods: It's a case control study. Sixty eight type 2 diabetic subjects of both the sex, aged between 35-65 years are included in the study, subjects are divided in to test and control group 34 each. Test group subjects did the yogasana and pranayama for a period of 6 months, 6 days in a week, 45-60 minutes daily under the supervision of a

qualified yoga teacher. Control group subjects are not on any specific exercise regimen. Both the group subjects are taking oral hypoglycaemic agents. HbA1c levels are measured using the Bio-Rad D-10™ haemoglobin A1c program and Magnetic Resonance Spectroscopy (MRS) is used in assessing the metabolite concentrations.

Statistical analysis: Analysis of data was done by using unpaired t-test. P-value for HbA1c level is <0.001, which is highly significant statistically. P-value for NAA was < 0.02 and for myoinositol was < 0.01, which are statistically significant.

Results : HbA1c levels in control and test group subjects are 7.7 ± 1.84 and 6.02 ± 0.46 respectively. NAA concentrations in the right dorsolateral frontal lobe of control and test group are 1.44 ± 0.15 and 1.54 ± 0.19 respectively. The ml concentrations in the right dorsolateral frontal lobe of control and test group are 0.61 ± 0.22 and 0.47 ± 0.24 respectively.

Conclusion: Yogasana and pranayama minimized the neuronal and glial cellular damage in test group, which is evident by minimal changes in right dorsolateral frontal lobe NAA and ml levels in type 2 diabetic subjects.

Keywords: Frontal lobe, Glycosilated haemoglobin, Yogasana and pranayama

INTRODUCTION

Globally 387 million people are suffering with diabetes out of which 90% of the cases are type 2 diabetic in nature, it is expected that 592 million people are going to suffer with diabetes by 2035 [1]. Type 2 diabetes mellitus is the result of non-responsiveness by the peripheral tissues to the secreted insulin or by the decreased secretion of insulin from the β cells of pancreas and the aetiological factor for type 2 diabetes is mainly obesity [2]. Reduced frontal lobe volumes are observed in obese type 2 adolescents [3]. Type 2 diabetes affects the functioning of central nervous system (cognition) by altering the brain metabolite concentrations [4]. Regular yogasana and pranayama enhances the functioning of central nervous system (cognition) [5]. Magnetic resonance spectroscopy (MRS) enables the identification and quantification of metabolites in given sample [6]. As frontal lobe is involved in executive functions and working memory, we are interested in finding, how the N-acetyl aspartate (NAA) & myoinositol (ml) concentrations are altered in the right dorsolateral frontal lobe of type 2 diabetic subjects and also to observe the effects of yogasana and pranayama on these metabolite concentrations. Change in the metabolite concentrations in the right dorsolateral frontal lobe will affect the memory [7,8]. Glycosilated haemoglobin (HbA1c) concentration is related to the development of complications and it reflects the mean plasma glucose levels over the last 2-3 months and has a minimum effect from the drugs that are influencing the glucose metabolism [9]. Absence or decreased

concentration of N-acetyl aspartate (NAA) is a sign of neuronal loss or degradation [6,10] and it might reflect intrinsic imbalance between excitatory versus inhibitory neurons [11].

Increased myoinositol (ml) concentration is observed with proliferation of glial cells or with increased glial-cell size, as found in inflammation [6]. Blood glucose levels will be elevated in type 2 diabetes [12]. In type 2 diabetic subjects, increased blood glucose levels will also increase the brain glucose levels and increased brain glucose levels will alter the brain metabolite concentrations [13]. The study is aimed to observe the HbA1c levels, NAA and ml concentrations in right dorsolateral frontal lobe in type 2 diabetic subjects who did the yogasana and pranayama and to compare the same with diabetic controls.

MATERIALS AND METHODS

The present study was approved by the Institutional Ethical Committee.

Inclusion criteria: Type 2 diabetes patients, both the sex are included, minimum duration of diabetes is 2 years, age between 35-65 years were included in the study.

Exclusion criteria: Smokers, alcohol consumers, any neuro-psychological disorders, recent history of major surgeries & those who are on insulin treatment were excluded. Sixty eight type 2 diabetic subjects were recruited in this study after taking the written

informed consent. Thirty four type 2 diabetic test group subjects did the yogasana and pranayama for 6 months, 6 days in a week, for a period of 45-60 minutes daily under the supervision of a qualified yoga expert in Yogi Vemana Yoga Research Institute in Hyderabad. Age and sex matched 34 type 2 diabetic subjects, who are not on any specific exercise regimen were included in the study as control group. Test group subjects did a set of yogasana followed by a set of pranayama which are listed in detail in [Table/Fig-1,2] respectively. Both the control and test group subjects are on oral hypoglycaemic agents. HbA1c levels were estimated by using the Bio-Rad D-10™ haemoglobin A1c program, and it is intended for the percent determination of haemoglobin A1c in human whole blood. It is based on high-performance liquid chromatography (HPLC). Samples will be injected in to the analytical cartridge after diluting them on D-10, programmed buffer gradient of increasing ionic strength will be delivered to the cartridge by the D-10 and here the haemoglobins are separated. The D-10 software and the exponentially modified Gaussian (EMG) algorithm will do the rest in quantifying the HbA1c levels [14]. The D-10 Haemoglobin A1c program is for use only with the Bio-Rad D-10 Haemoglobin Testing

S. No	Name of Yogasana	Duration
1	Dhanurasana	1/2 minute to one minute for the pose being maintained, adding 1/2 minute per week
2	Naukasana	2 - 4 turn of each, the pose being maintained for ten seconds adding one turn each, every fortnight
3	Arthamasthendrasana	¼ minute to one minute for each side, adding ¼ minute per week
4	Bhujangasana	2 - 4 turn of each, the pose being maintained for ten seconds adding one turn each, every fortnight
5	Shavaasana / Makarasana	3 turn of each, the pose being maintained for 30 seconds

[Table/Fig-1]: List of Yogasana

S. No	Name of Pranayama	Duration
1	Anuloma-viloma	2-5 minutes
2	Surya anuloma-viloma	5 minutes
3	Chandra anuloma-viloma	5 minutes
4	Nadishuddhi pranayama	10 minutes

[Table/Fig-2]: List of Pranayama

System [14]. Magnetic Resonance Spectroscopy was performed in the Department of Radiology in MediCiti Institute of Medical Sciences. Metabolite concentrations in right dorsolateral frontal lobe were estimated with 1.5 Tesla Phillips magnetic resonance imaging machine.

Acquisition parameters

Right dorsolateral frontal lobe NAA and ml concentrations were estimated with Magnetic Resonance Spectroscopy (MRS) using a

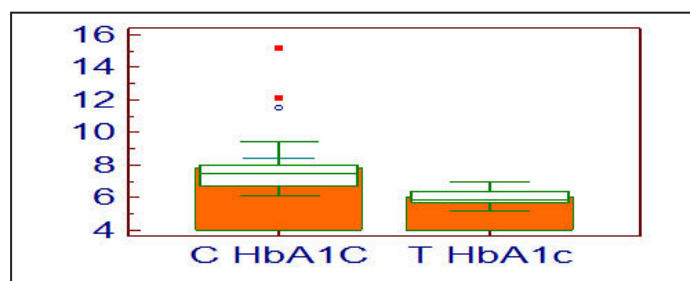
single voxel technique. Single voxel point resolved spectroscopy (PRESS) sequence was used for volume of interest (VOI) localization (TR/TE =1800 msec/36msec; NSA 96; Spectral band width 1000; scan time 3 min 25 sec) [6]. Based on the axial T2-weighted image, a voxel (20×20×15 mm) was positioned in the frontal white matter, avoiding the cortex and lateral ventricle. MOIST was used for water suppression in spectroscopy sequences and spectral correction was done [6]. Only peaks of NAA and ml were estimated but not the peaks of other metabolites like choline, glutamate, glutamine, acetylcholine etc.

STATISTICAL ANALYSIS

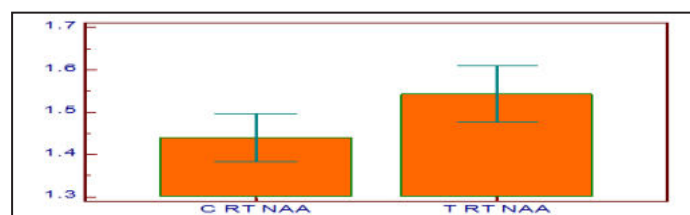
The results were analysed by using Med Calc Statistical Software version 12.7.8 (Med Calc Software bvba, Ostend, Belgium; <http://www.medcalc.org>; 2014). Unpaired student's t-test was used in finding the metabolite concentration between test and control groups. A p-value <0.05 was considered as statistically significant.

RESULTS

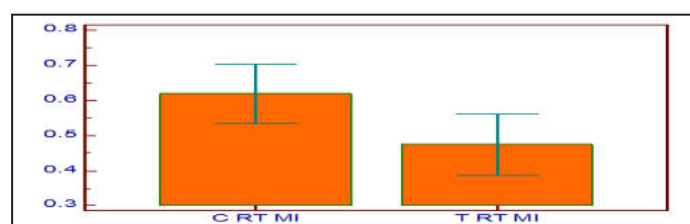
[Table/Fig-3] shows the HbA1c levels in control and test group subjects and they are 7.7 ± 1.84 and 6.02 ± 0.46 respectively. The



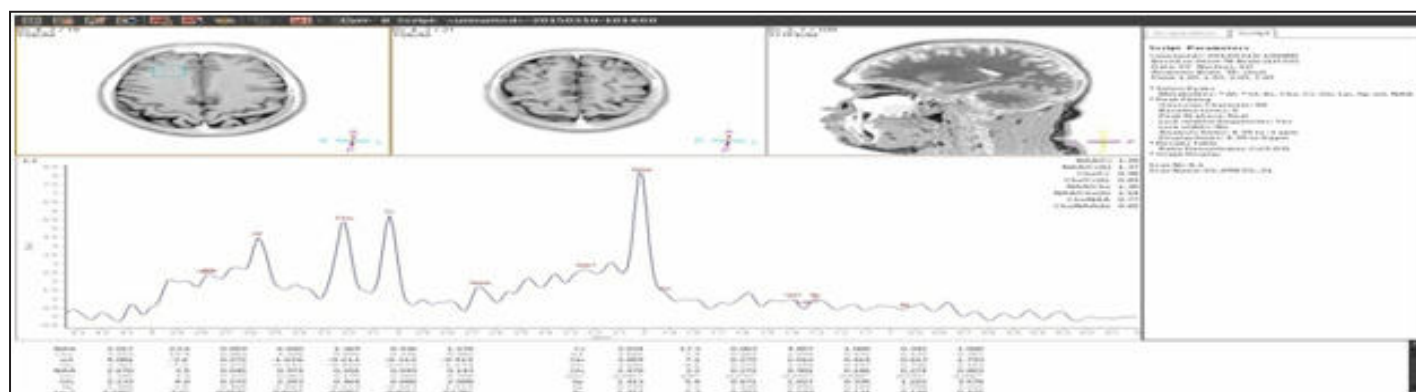
[Table/Fig-3]: HbA1c levels in control and test group subjects



[Table/Fig-4]: N-acetyl aspartate concentration in right dorsolateral frontal lobe in control and test group



[Table/Fig-5]: Myo-inositol concentration in right dorsolateral frontal lobe in control and test group



[Table/Fig-6]: Right dorsolateral frontal lobe MRS in a subject

p-value for HbA1c is <0.001 which is highly significant statistically. [Table/Fig-4] shows the NAA concentrations in the right dorsolateral frontal lobe of control and test group, and they are 1.44 ± 0.15 and 1.54 ± 0.19 respectively. The p-value for NAA is 0.02. [Table/Fig-5] shows the ml concentrations in the right dorsolateral frontal lobe of control and test group, and they are 0.61 ± 0.22 and 0.47 ± 0.24 respectively. The p-value for ml is 0.01. [Table/Fig-6] is the graphical representation of Magnetic Resonance Spectroscopy (MRS) of right dorsolateral frontal lobe in a given subject, showing the peaks of the different metabolites.

DISCUSSION

High HbA1c levels and low NAA concentration are observed in controls when compared to yogasana and pranayama group that are reflected in [Table/Fig-3,4] respectively. Low concentration of NAA in control group is because of decreased neuronal viability [6,11]. It indicates that neuronal loss is the cause for low concentration of NAA in type 2 diabetes, this neuronal loss is the result of osmolyte imbalance and this is due to accumulation of advanced glycosylated end products [6]. Myoionositol concentration is more in controls than the yogasana and pranayama group as shown in [Table/Fig-5]. Increased ml concentration is an indication of myelin degradation, proliferation of glial cells or with increased glial cell size i.e., inflammation [15]. Increased HbA1c levels in controls will increase the right dorsolateral frontal lobe glucose levels [13]. High glucose levels in right dorsolateral frontal lobe will increase the myoionositol concentration in type 2 diabetic subjects [16-18] and decreases the N-acetyl aspartate (NAA) concentration [13,16]. Myoionositol (ml) concentration is more in diabetic controls than the yogasana and pranayama group and this is either because of increased intra neuronal glucose which have converted in to inositol 1-phosphate which in turn converted in to ml [19] or because of increased intracellular osmotic pressure which have led the increase in ml levels [6]. Where as in a study by Sinha et al., there is no significant change in the ml concentration in right frontal lobe although increased right frontal lobe glucose was observed [13]. Further studies are required in analysing this contradicting variation in frontal lobe myoionositol (ml) in type 2 diabetes. In test group yogasana and pranayama normalized or near normalized the HbA1c levels either by increasing the peripheral uptake of glucose or by increasing insulin secretion by beta cells of islets of Langerhas [20]. Increased uptake of peripheral glucose or increased insulin secretion in test group is because yogasana and pranayama stimulate the parasympathetic nervous system resulting in normal HbA1c levels. Normal or near normal plasma HbA1c levels might have kept the right dorsolateral frontal lobe glucose levels in control. Normal right dorsolateral frontal lobe glucose levels maintained the near normal N-acetyl aspartate (NAA) and myoionositol (ml) concentrations in test group subjects. Thus yogasana and pranayama are helpful in stabilizing the plasma HbA1c levels [20-33] and stable HbA1c levels will keep the N-acetyl aspartate (NAA) and myoionositol (ml) concentrations near normal values in right dorsolateral frontal lobe. Outcome of this study is, yogasana and pranyama should be advised to type 2 diabetic patients for maintaining the normal functioning of the right frontal lobe by keeping the normal N-acetyl aspartate (NAA) and myoionositol (ml) concentrations.

STUDY LIMITATIONS AND FUTURE PROSPECTS

We could not measure the type and quantity of food the subjects were taking. Measuring the stress levels also help in better understanding the role of yogasana and pranayama on HbA1c levels. Difficult to record the number of hyperglycaemic and hypoglycaemic attacks per day in diabetic subjects for assessing the effects of the same on brain metabolites. All the brain metabolite concentrations should be explored in multiple lobes of the brain in type 2 diabetes. By knowing the degree of variations of these metabolites in different

lobes of the brain with HbA1c levels one can take the prophylactic measures.

CONCLUSION

In test group, yogasana and pranayama reduced the HbA1c levels to near upper limit of the normal values and this near normal HbA1c level is restricted the wide variations of right dorsolateral frontal lobe N-acetyl aspartate (NAA) and myoionositol (ml) concentrations, which are seen in diabetic controls.

ACKNOWLEDGEMENT

Research reported in this publication was conducted by scholars at the Fogarty International Center of the NIH training program under Award Number D43 TW 009078. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Health.

REFERENCES

- [1] International Diabetes Federation. Diabetes update-2014. <http://www.idf.org/diabtesatlas/update-2014> (accessed 29 November 2014).
- [2] World Health Organization. Diabetes Fact sheet No.312. <http://www.who.int/mediacentre/factsheets/fs312/en/> (accessed 10th October 2013).
- [3] Bruehl H, Sweat V, Tirsi A, Shah B, Convit A. Obese Adolescents with Type 2 Diabetes Mellitus Have Hippocampal and Frontal Lobe Volume Reductions. *Neurosci Med*. 2011;2(1): 34-42.
- [4] Rajani S, Reddy IY, Archana R. Effect of Type 2 Diabetes Mellitus on Brain Metabolites by Using Proton Magnetic Resonance Spectroscopy - A Systematic Review. *Int J Pharm Bio Sci*. 2014;5(4):1118-23.
- [5] Rajani S, Reddy IY, Kumar S, Archana R. Study of cognition in type 2 diabetes with yoga asana and pranayama. *RJPBCS*. 2013;3(2):1637-41.
- [6] Bertholdo D, Watcharakorn A, Castillo M. Brain Proton Magnetic Resonance Spectroscopy: Introduction and Overview. *Neuroimaging Clin N Am Elsevier Inc*. 2013;23(3):359-80.
- [7] Van Harten B, De Leeuw FE, Weinstein HC, Scheltens P, Biessels GJ. Brain Imaging in Patients with Diabetes, A Systematic review. *Diabetes Care*. 2006;29(11):2539-48.
- [8] Tiehuis A, Van Der Meer F, Mali W, Plezier M, Biessels GJ, Kappelle J. MR spectroscopy of cerebral white matter in type 2 diabetes; No association with clinical variables and cognitive performance. *Neuroradiology*. 2010;52(2):155-61.
- [9] Lapolla A, Mosca A, Fedele D. The general use of glycated haemoglobin for the diagnosis of diabetes and other categories of glucose intolerance: Still a long way to go. *Nutrition, Metabolism & Cardiovascular Diseases*. 2011;21:467-75.
- [10] Cecil KM. Proton Magnetic Resonance Spectroscopy Technique for the Neuroradiologist. *Neuroimag Clin N Am*. 2013;23:381-92.
- [11] Grachev ID, Kumar R, Ramachandran TS, Szeverenyi NM. Cognitive interference is associated with neuronal marker N-acetyl aspartate (NAA) in the anterior cingulate cortex: an in vivo 1H-MRS study of the Stroop Color- Word task. *Molecular Psychiatry Nature Publishing Group*. 2001;6:529-39.
- [12] Christopher Haslett. Davidson's Principles and Practice of Medicine, 19 ed.: Churchill Livingstone; 2002.
- [13] Sinha S, Ekka M, Sharma U, Raghunandan P, Pandey RM, Jagannathan NR. Assessment of changes in brain metabolites in Indian patients with type-2 diabetes mellitus using proton magnetic resonance spectroscopy. *BMC Research Notes*. 2014;7(41):2-7.
- [14] Pack R. Haemoglobin A1c Program Instruction Manual. United States, Bio-Rad Laboratories, Inc., Hercules, CA 94547; 1-28 p.
- [15] Geissler A, Frund R, Scholmerich J, Feuerbach S, Zietz B. Alterations of cerebral metabolism in patients with diabetes mellitus studied by proton magnetic resonance spectroscopy. *Exp Clin Endocrinol Diabetes*. 2003; 111:421-27.
- [16] Sahin I, Alkan A, Keskin L, Cikim A, Karakas HM, Firat AK. Evaluation of in vivo cerebral metabolism on proton magnetic resonance spectroscopy in patients with impaired glucose tolerance and type 2 diabetes mellitus. *J Diabetes Complications*. 2008;22(4):254-60.
- [17] Ajilore O, Haroon E, Kumaran S, Darwin C, Binesh N, Mintz J. Measurement of brain metabolites in patients with type 2 diabetes and major depression using proton magnetic resonance spectroscopy. *Neuropsychopharmacology*. 2007;32:1224-31.
- [18] Haroon E, Watari K, Thomas A, Ajilore O, Mintz J, Elderkin-Thompson V. Prefrontal myo-inositol concentration and visuospatial functioning among diabetic depressed patients. *Psychiatry Res*. 2009;171(1):10-19.
- [19] Van der Graaf M, Janssen SWJ, van Asten JJ, Hermus ARMM, Sweep CGJ, Plikkemaat JA. Metabolic profile of the hippocampus of Zucker Diabetic Fatty rats assessed by in vivo 1H magnetic resonance spectroscopy. *NMR Biomed*. 2004;17:405-10.
- [20] Sahay BK. Role of yoga in diabetes. *J Assoc Physicians India*. 2007;55:121-26.

- [21] Shembekar AG, Kate SK. Yoga exercises in the management of diabetes mellitus. *J Diabetic Assoc India*. 1980;20:167-71.
- [22] Nagarathna R, Nagendra HR. Proceedings of the Ninth Annual Conference of the IEEE Engineering in Medicine and Biology Conference. New York, USA: IEEE, Integrated approach of yoga therapy in the management of diabetes mellitus; 1987; pp. 1593-94.
- [23] Gupta SM. Modern medicine and yoga. *J Intern Med India*. 2001;4:155-56.
- [24] Malhotra V, Singh S, Tandon OP, Madhu SV, Prasad A, Sharma SB. Effect of Yoga asanas on nerve conduction in type 2 diabetes. *Indian J Physiol Pharmacol*. 2002;46(3):298-306.
- [25] Manyam BV, Sahay BK, Sahay RK. Lifestyle modification in management of diabetes mellitus. *J Indian Med Assoc*. 2002;100(3):178-80.
- [26] Stevens DL. The use of complementary and alternative therapies in diabetes. *Clin Fam Prac*. 2002;4:911-28.
- [27] Kaplan-Mayer G. Get moving with yoga. *Diabetes Self Manag*. 2003; 20(4):28,31-3.
- [28] Manyam BV. Diabetes mellitus, Ayurveda, and yoga. Comment. *J Altern Complement Med*. 2004;10(2):223-25.
- [29] Khalasa SB. Yoga as a therapeutic intervention: a bibliometric analysis of published research studies. *Indian J Physiol Pharmacol*. 2004;48(3):269-85.
- [30] Nayak NN, Shankar K. Yoga: a therapeutic approach. *Phys Med Rehabil Clin N Am*. 2004;15:783-98.
- [31] Singh S, Malhotra V, Singh KP, Madhu SV, Tandon OP. Role of Yoga in modifying certain cardiovascular functions in type 2 diabetic patients. *J Assoc Physicians India*. 2004;52:203-06.
- [32] Malhotra V, Singh S, Tandon OP, Sharma SB. The beneficial effect of yoga in diabetes. *Nepal Med Coll J*. 2005;7(2):145-47.
- [33] Dham S, Shah V, Hirsch S, Banerji MA. The role of complementary and alternative medicine in diabetes. *Curr Diab Rep*. 2006;6(3):251-58.

PARTICULARS OF CONTRIBUTORS:

1. Assistant Professor, Department of Physiology, MediCiti Institute of Medical Sciences, Hyderabad, India.
2. Associate Professor, Department of Physiology, MediCiti Institute of Medical Sciences, Hyderabad, India.
3. Professor, Department of Physiology, MediCiti Institute of Medical Sciences, Hyderabad, India.
4. Professor, Department of Physiology, MediCiti Institute of Medical Sciences, Hyderabad, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Yogananda Reddy,
Associate Professor, Department of Physiology, MediCiti Institute of Medical Sciences, Hyderabad-5014011, India.
E-mail : Yoga4udotcom@yahoo.co.in

Date of Submission: **Mar 23, 2015**
Date of Peer Review: **Apr 24, 2015**
Date of Acceptance: **Jun 17, 2015**
Date of Publishing: **Jul 01, 2015**

FINANCIAL OR OTHER COMPETING INTERESTS: None.