YENEPOYA
(DEEMED TO BE UNIVERSITY)
Recognized under Sec 3(A) of the UGC Act 1956
Accredited by NAAC with 'A' Grade

Details of the Collaborative Activity

2020-21

Name of the Collaborating Institute: Government Medical College, Srinagar, Jammu and Kashmir, India.

Name of the Collaborating Department: Department of Biochemistry, Yenepoya Medical College.

Joint Research and Publications

Sadaf Ali, Department of Biochemistry, Shiekh Amir, Senior Resident, Department of Forensic Medicine from Government Medical College, Srinagar and Dr. Nivedita L., Professor, Department of Biochemistry, Yenepoya Medical College published joint publications through collaborative research as follows;

1. Sadaf Ali, Shiekh Amir, Nivedita L. Serum Fluorescence of advanced glycation end products: A potential screening tool to distinguish between diabetic patients with and without micro vascular complications. *Journal of Clinical and Diagnostic Research*, 2020; 14(11): BC06-BC09.
2. Sadaf Ali, Shiekh Amir, Nivedita L. Diabetes mellitus and thyroid dysfunction: A double edged sword. *International Journal of Scientific Research* Vol-9, Issue 8, August 2020.
3. Sadaf Ali, Shiekh Amir, Nivedita L. Correlation of serum fluorescence of advanced glycation end products with diabetes duration and glycemic control in type 2 diabetic patients. *Journal of Biomedical Research and Therapy* 2020: 7(8):3933-38

ATTESTED

Dr. Gangadhara Somayaji K S
Registrar
Yenepoya (Deemed to be University)
University Road, Deralakatte
Mangalore 575 018, Karnataka.

Original Article

DOI: 10.7860/JCDR/2020/45484.14191

Biochemistry Section

Serum Fluorescence of Advanced Glycation End Products: A Potential Screening Tool to Distinguish Between Diabetic Patients with and without Microvascular Complications

SADAF ALI[1], NIVEDITA L RAO[2]

ABSTRACT

Introduction: Enhanced formation and accumulation of Fluorescent Advanced Glycation End products (F-AGEs) in diabetes mellitus have been linked to increased risk of developing the associated vascular complications.

Aim: To evaluate the potential of serum fluorescence levels of F-AGEs as screening tools to distinguish between type 2 diabetic patients with and without microvascular complications such as retinopathy, neuropathy.

Materials and Methods: This cross-sectional study was conducted between June 2016 and June 2017, included 95 type 2 diabetic patients with more than 1 year of diabetes duration. Fasting blood glucose, glycated haemoglobin and total protein levels were estimated by automated methods. Serum F-AGEs were estimated by using a simple spectrofluorometric method. Microvascular complications due to diabetes mellitus were studied in each patient from medical records data on fundus examination for retinopathy and touch, vibration sensation detection for neuropathy. Diabetic patients were categorised into two groups as those without microvascular complications and those with microvascular complications-retinopathy and neuropathy. Statistical tests used for comparisons between groups were chi-square test for gender distribution, independent t-test for other parameters and Pearson's correlations. The p-value <0.05 indicated significant difference between variables.

Results: Mean age of the population was 55.1±5.3 years. Diabetic patients with microvascular complications (n=26) in the form of retinopathy, neuropathy had significantly higher levels of serum F-AGEs with mean 7.4±1.8 AU/g protein compared with diabetic patients without complications with mean value 1.5±0.7 AU/g protein (p<0.01).

Conclusion: Two categories of serum fluorescent AGE values, without overlap, could be distinguished between diabetic patients with and without complications. Measurement of serum F-AGEs products has the potential to emerge as a simple, valuable screening tool to distinguish between diabetic patients with and without microvascular complications.

Keywords: Diabetes mellitus, Neuropathy, Retinopathy, Spectrofluorometry

INTRODUCTION

Diabetes mellitus is a metabolic disease characterised by elevated blood glucose levels or hyperglycaemia resulting from defects in insulin secretion, insulin action or both. Chronic hyperglycaemia is associated with microvascular complications due to damage to small blood vessels and macrovascular due to damage to larger blood vessels. Microvascular complications include damage to retina of eyes (retinopathy), kidneys (nephropathy) and nerves (neuropathy). Macrovascular complications include cardiovascular diseases, peripheral vascular and cerebrovascular diseases. These conditions can lead to end organ damage such as visual impairment, kidney disease, nerve damage, diabetic foot, heart disease, and stroke [1]. Diabetic macroangiopathy, atherosclerosis secondary to diabetes mellitus, cause cerebro-cardiovascular diseases which are major causes of death in diabetic patients. Studies have revealed that major underlying biochemical reactions are overproduction of reactive oxygen species, increased formation of AGEs and activation of the AGEs receptors [2,3].

AGEs are produced through the non-enzymatic glycation reactions and also by oxidation of proteins, lipids and nucleic acids. Hyperglycaemic condition in diabetes mellitus leads to their enhanced formation. Enhanced formation and accumulation of AGEs have been linked to increased risk for both macro- and microvascular complications associated with diabetes mellitus [4,5].

Advanced glycation is the non-enzymatic process whereby the carbonyl (aldehyde or ketone) of reducing sugars like glucose react nonenzymatically with lysine and N-terminal amino groups in different proteins, lipoproteins, and nucleic acids, that leads to the formation of early glycation products via the Maillard reaction. These go through further rearrangements leading to the formation of various reactive intermediate products including- dicarbonyls or oxoaldehydes- dicarbonyls react with amino groups of intracellular and extracellular proteins to form AGEs, a heterogeneous class of stable and irreversible covalent adducts. Pentosidine, argpyrimidine, many other AGEs have intrinsic florescence and hence tissue and plasma fluorescence may be used as markers to estimate AGE accumulation. Other AGEs such as Carboxymethyllysine (CML) and pyralline do not have properties of fluorescence [6].

AGEs are believed to modulate cellular processes by binding to specific cell surface receptors present on the surface of all cells relevant to processes of atherosclerosis triggering oxidative stress, inflammation and apoptosis [7,8].

In addition to endogenous AGEs, exogenous AGEs are produced by reactions between sugars and proteins in ingested foods which may be absorbed into the circulation. AGE content is high in cooked and processed foods, especially those rich in proteins, fat, and sugar. It is considered that dietary AGEs are similar to endogenous AGEs with regard to their pro-oxidant, pro-inflammatory, and signalling properties [9].

Elevated serum level of AGEs, RAGE and pentosidine have been reported in Tunisian patients with diabetic retinopathy [10]. The underlying mechanisms involving AGEs in the pathogenesis

retinopathy too [4]. A standardised approach to quantify all varieties of AGEs simultaneously remains unestablished because of the vast heterogeneity of the AGEs.

The spectrofluorometric estimation method used in the present study, is relatively simple to perform, less laborious and suitable for easy screening purpose in primary care setting, compared to other methods. It can distinguish between diabetic patient with and without microvascular complications as demonstrated in the present, as well as in other reported studies. Furthermore, use of the portable spectrofluorometer, may facilitate point-of-care testing of diabetic patients for serum F-AGEs.

Limitation(s)

Significant variation was present between the mean age of diabetic patients with and without microvascular complications, which may be a confounding factor. The mean diabetes duration of patients was not calculated in the present study.

CONCLUSION(S)

Measurement of serum fluorescence of AGEs using the simple spectrofluorometric method has the potential to become a useful, reliable, screening test to detect diabetic patients with hyperglycaemia induced microvascular complications. Risk stratification for vascular complications and monitoring response to therapy are the other potential outcomes of measuring serum fluorescent AGEs in diabetic patients.

Acknowledgement

The authors of this study are thankful to Dr. Ravi Vaswani, Professor, Dept of General Medicine, Yenepoya Medical College Hospital for helping in patient selection for the study. The authors also thank Ms. Megha, Statistician at Yenepoya Medical College Hospital (deemed to be University) for conducting statistical analysis of data.

REFERENCES

[1] Ozougwu JC, Obimba KC, Belonwu CD, Unakalamba CB. The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. J Physiol Pathophysiol. 2013;4(4):46-57.
[2] Katakami N. Mechanism of development of atherosclerosis and cardiovascular disease in diabetes mellitus. J Atheroscler Thromb. 2018;25(1):27-39.
[3] Hegab Z, Gibbons S, Neyses L, Mamas AM. Role of advanced glycation end products in cardiovascular disease. World J Cardiol. 2012;4(4):90-102.
[4] Maza MP, Garrido F, Escalante N, Leiva L, Barrera G, Schnitzler S, et al. Fluorescent advanced glycation end-products (ages) detected by spectro-photofluorimetry, as a screening tool to detect diabetic micro vascular complications. J Diabetes Mellitus. 2012;2(2):221-26.
[5] Coughlan MT, Patel SK, Jerums G, Penfold SA, Nguyen TV, Sourris KC, et al. Advanced glycation urinary protein-bound biomarkers and severity of diabetic nephropathy in man. Am J Nephrol. 2011;34(4):347-55.
[6] Tan ALY, Forbes JM, Cooper ME. AGE, RAGE and ROS in diabetic nephropathy: Semin Nephrol. 2007;27(2):130-43.
[7] Nowotny K, Jung T, Hohn A, Weber D, Grune T. Advanced glycation end products and oxidative stress in type 2 diabetes mellitus. Biomolecules. 2015;5(1):194-222.
[8] Singh VP, Bali A, Singh N, Jaggi AS. Advanced glycation end products and diabetic complications. Korean J Physiol Pharmacol. 2014;18(1):01-14.
[9] Uribarri J, Cai W, Peppa M, Goodman S, Ferrucci L, Striker G, et al. Circulating glycotoxins and dietary advanced glycation end products: Two links to inflammatory response, oxidative stress, and aging. J Gerontol A Biol Sci Med Sci. 2007;62(4):427-33.
[10] Kerkeni M, Saidi A, Bouzidi H, Yahya SB, Hammami M. Elevated serum level of AGEs, RAGE and pentosidine in Tunisian patients with severity of diabetic retinopathy. Microvasc Res. 2012;84(3):378-83.
[11] Takeuchi M, Takino J, Yamagishi S. Involvement of the toxic AGEs (TAGE)-RAGE system in the pathogenesis of diabetic vascular complications: A novel therapeutic strategy. Curr Drug Targets. 2010;11(11):1468-82.
[12] Guerin-Dubourg A, Cournot M, Planesse C, Debussche X, Meilhac O, Rondeau P, et al. Association between fluorescent advanced glycation end-products and vascular complications in type 2 diabetic patients. Biomed Res Int. 2017;2017:7989180. doi: 10.1155/2017/7989180.
[13] Folli F, Corradi D, Fanti P, Davalli A, Paez A, Giaccari A, et al. The role of oxidative stress in the pathogenesis of type 2 diabetes mellitus micro- and macro vascular complications: Avenues for a mechanistic-based therapeutic approach. Curr Diabetes Rev. 2011;7(5):313-24.
[14] Raposeiras-Roubín S, Rodiño-Janeiro BK, Paradela-Dobarro B, Grigorian-Shamagian L, García-Acuña JM, Aguiar-Souto P, et al. Fluorescent advanced glycation end products and their soluble receptor: The birth of new plasmatic biomarkers for risk stratification of acute coronary syndrome. PLoS One. 2013;8(9). doi.org/10.1371/journal.pone.0074302.
[15] Aragno M, Mastrocola R. Dietary sugars and endogenous formation of advanced glycation end products: Emerging mechanisms of disease. Nutrients. 2017;9(4):385.
[16] Ashraf JM, Ahmad S, Choi I, Ahmad N, Farhan M, Tatyana G, et al. Recent advances in detection of AGEs: Immunochemical, bioanalytical and biochemical approaches. IUBMB Life. 2015;67(12):897-913.
[17] Munch G, Keis R, Wessels A, Riederer P, Bahner U, Heidland A, et al. Determination of advanced glycation end products in serum by fluorescence spectroscopy and competitive ELISA. Eur J Clin Chem Clin Biochem. 1997;35(9):669-77.

PARTICULARS OF CONTRIBUTORS:
1. Senior Resident, Department of Biochemistry, Government Medical College, Srinagar, Jammu and Kashmir, India.
2. Professor, Department of Biochemistry, Yenepoya Medical College, Mangalore, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Nivedita L Rao,
Department of Biochemistry, Yenepoya Medical College, Yenepoya (Deemed to be University), Deralakatte, Mangalore, Karnataka, India.
E-mail: nlrdr@yahoo.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jun 15, 2020
- Manual Googling: Sep 01, 2020
- iThenticate Software: Oct 16, 2020 (21%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: Jun 14, 2020
Date of Peer Review: Jul 23, 2020
Date of Acceptance: Sep 01, 2020
Date of Publishing: Nov 01, 2020

ATTESTED
Dr.Gangadhara Somayaji K.S.
Registrar
Yenepoya(Deemed to be University)
University Road, Deralakatte,
Mangalore- 575 018, Karnataka

ORIGINAL RESEARCH PAPER

Volume - 9 | Issue - 8 | August - 2020 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

DIABETES MELLITUS AND THYROID DYSFUNCTION: A DOUBLE EDGED SWORD.

Biochemistry

Dr. Sadaf Ali Senior Resident, Department of Biochemistry, Faculty of Medicine, Government Medical College, Srinagar.

Dr. Nivedita L Rao Professor, Department of Biochemistry, Faculty of Medicine, Yenepoya Medical College and Hospital Yenepoya University, Deralakatte, Mangalore, India.

Dr. Shiekh Amir* Senior Resident, Department of Forensic Medicine, Faculty of Medicine, Government Medical College, Srinagar. *Corresponding Author

ABSTRACT

Interlinking mechanism of thyroid disorders in diabetes mellitus has been suggested by various studies. Inter-connections at the molecular level such as the common signalling pathways explain the linkage. Untreated and undiagnosed thyroid disorders may affect insulin sensitivity and resistance thereby hampering glycaemic control in diabetic patients. Aims and objectives: To know thyroid functioning and spectrum of dysfunctions in type 2 diabetic patients. Materials and methods: Serum samples were obtained from 95 diabetic patients of both genders. Patients who visited the Department of Medicine of Yenepoya Hospital for treatment were taken for the purpose of this study. Some of the patients were on treatment with oral hypoglycaemic drugs and some on insulin or both. Patients were divided into two groups based on duration of disease. Levels of T3, T4 and TSH were measured in all the patient samples using VITROS 5600 analyser. Results: In our study population hypothyroidism was more common in diabetic females when compared to the diabetic male subjects. Similar results were seen in sub-clinical hypothyroidism; which was more commonly found in female patients. Possible reason for this may be attributed to hormonal imbalance in women.

KEYWORDS

Thyroid, hypothyroidism, diabetic patients.

INTRODUCTION:

The two most common endocrine disorders that come across during clinical practice are thyroid disorders and diabetes mellitus. Different types of thyroid abnormalities are associated with diabetes mellitus. There are many studies suggestive of conflicting findings between thyroid dysfunction in diabetic patients. According to some studies, the prevalence of thyroid dysfunction among type two diabetic patients was very high about 30% with sub clinical hypothyroidism being most common.3,4 Other studies done in different populations suggested thyroid dysfunction as a common complication in diabetic patients attributed mainly to complex interlinking biochemical, genetic, and hormonal malfunctions that reflects this patho-physiological association.5 Thyroid gland is located immediately below the larynx on each side, anterior to the trachea in our body. It is one of the largest endocrine glands weighing about 15 to 20 grams in adults. Thyroid gland mainly secretes two major hormones, thyroxin and tri-iodothyronine (commonly called T4 and T3). Both of these hormones profoundly affect metabolic rate of the body. Secretion of thyroid hormones in the circulation is mainly controlled by levels of thyroid stimulating hormone (TSH). 1 TSH is formed by anterior pituitary gland. Thyroxine forms about 93% and tri iodothyronine forms 7% of the metabolically active thyroid hormones. Thyroxine is eventually converted to tri iodothyronine and is about four times as potent as thyroxine. In the blood however, it is present in smaller quantities and persists for a much shorter time than thyroxine.[2]

Thyroid hormones mainly regulate pancreatic function and carbohydrate metabolism while as diabetes mellitus leads to changes in normal thyroid functioning.17 Variations in Hypothalamic-pituitary-thyroid axis also produce significant metabolic disturbances. Thyroid function is influenced by diabetes mellitus at the level of hypothalamic control of TSH release and at tissue level; where conversion of T4 to T3 takes place. Hyperglycemias reduce hepatic concentration of enzymes like T4-5 de-iodinase, decrease serum concentration of T3, and raised levels of reverse T3 and low, normal, or high level of T[4].

The overall prevalence of thyroid dysfunction among type 2 DM patients is relatively higher on many occasions. Diabetic patients should be consequently screened for thyroid dysfunction in order to reduce the mortality rate.5-8 Defective insulin secretion leads to various metabolic changes in T2DM, varying from hyperglycaemia to dyslipidemias. Former is due to defective insulin-stimulated uptake of glucose and up regulated hepatic glucose production and later attributed to impaired homeostasis of fatty acids and accumulation of triglyceride's and lipoproteins.3, 5 Hence, routine screening for thyroid dysfunction is suggested in diabetic patients, which will help in early diagnosis and treatment thereby improving their quality of life and eventually reduction in the morbidity rate.[3,4]

Materials and methods:

Diabetic patients in the age group of 30 to 60 years who attended Yenepoya Hospital participated for the purpose of this study. Type 2 diabetic patients were considered for the purpose of this study. Permission was granted for conducting this study from the Institutional Ethical Committee. Informed written consent was taken from every patient. A Cross sectional study was carried. Blood samples from 95 diabetic patients both male and female attending the Department of Medicine of Yenepoya Medical College and Hospital were obtained. Patients were grouped into two: Group 1 consisted of patients who had less than ten years of duration of diabetes mellitus and group 2 consisted of patients with more than ten years of type 2 DM. Fasting venous blood samples were collected from OPD patients as well as from the admitted patients. Exclusion and inclusion criteria were fulfilled from each patient. Patients having cardiac failure, pulmonary diseases, renal failure, cancer and alcoholism were excluded from the study.

Clinical and Biochemical measurements:

Fasting blood sample was obtained from each patient with all aseptic precautions and the following parameters were measured: T3, T4, TSH, and FBS. The VITROS GLU slide method was performed on VITROS 250/350/950 and 5, 1 FS Chemistry systems and the VITROS 5600 integrated system for the estimation of blood sugar. A competitive immunoassay technique using the VITROS 5600 Integrated System (intellicheck technology) was used for measuring the levels of T3, T4 and TSH. The bound HRP conjugate was measured by a luminescent reaction.

Results and Discussion:

Table 1. Thyroid status in diabetic study groups:

		Thyroid status			Total	p value
		Euthyroid	Hypothyroid	Sub clinical hypothyroidism		
Group 1 DM duration <10 years	N	27	14	5	46	.944

Biomedical Research and Therapy, 7(8):3933-3938

Open Access Full Text Article

Original Research

Correlation of serum fluorescence of advanced glycation end products with diabetes duration and glycemic control in type 2 diabetic patients

Sadaf Ali[1], Nivedita L. Rao[2,*]

Use your smartphone to scan this QR code and download this article

ABSTRACT

Introduction: Advanced glycation end products (AGEs) and diabetes duration have roles in the development of the vascular complications associated with morbidity and mortality in diabetic patients. The present study was conducted to estimate and find the association between serum fluorescence levels of advanced glycation products with diabetes duration and glycemic control in type 2 diabetic patients. **Methods**: 46 patients who had diabetic duration of less than ten years and 49 patients with duration more than ten years were included in the study. Serum fluorescence of AGEs was measured using a simple spectrofluorometric method. Correlations of AGEs with diabetes duration, fasting glucose, and glycated hemoglobin levels were analyzed. The incidence of microvascular complications in patients of both groups was examined. **Results**: Significantly higher serum fluorescent AGE levels ($p < 0.001$) and higher incidence of microvascular complications ($p = 0.000$) were found in diabetic patients who had diabetes duration of more than ten years, poorer glycemic control and higher age. Serum levels of fluorescent AGEs showed significant positive correlations with duration of diabetes mellitus, glycated haemoglobin and fasting glucose levels. **Conclusion**: Screening patients for AGEs, intensive glycemic control, and therapeutic strategies that target molecular mechanisms involving advanced glycation end products are warranted in older patients with longer diabetes duration to minimize their risk of developing complications.

Key words: Serum fluorescent advanced glycation end products, diabetes duration, glycemic control, correlations

[1]Department of Biochemistry, Government Medical College, Srinagar, Jammu and Kashmir, India

[2]Department of Biochemistry, Yenepoya Medical College, Yenepoya (deemed to be University), Mangalore, Karnataka, India

Correspondence

Nivedita L. Rao, Department of Biochemistry, Yenepoya Medical College, Yenepoya (deemed to be University), Mangalore, Karnataka, India

Email: nlrdr@yahoo.com

History

- Received: Jul 08, 2020
- Accepted: Aug 22, 2020
- Published: Aug 31, 2020

DOI : 10.15419/bmrat.v7i8.623

Check for updates

BioMedPress
The Open Access Publisher

INTRODUCTION

Diabetes mellitus, a group of metabolic diseases characterized by hyperglycemia, is a major national and global public health problem. Current global estimates indicate that the disease has affected 463 million people and is set to escalate to 578 million by the year 2030[1]. India has an estimated number of 77 million adults with diabetes, making India the second most affected country in the world after China[1,2]. Morbidity and mortality associated with the disease are mainly due to the resulting microvascular and macrovascular complications[3]. Long-term damage, dysfunction, and failure of various organs are associated with chronic hyperglycemia of diabetes. Chronic microvascular complications of diabetes include retinopathy, nephropathy and neuropathy[4–6]. Diabetic retinopathy is a common microvascular complication of diabetes, estimated to be responsible for 10,000 new cases of blindness every year in the United States alone[7]. Chronic macrovascular complications of diabetes are cardiovascular diseases, as well as peripheral vascular and cerebrovascular diseases. Patients with diabetes are at 2-4 times increased risk of coronary heart disease, peripheral vascular disease, and related deaths than those in the general population[8].

The impact of age, age at diagnosis of diabetes, and diabetes duration on subsequent vascular complications has been investigated in some studies but have yielded a variety of results. A positive association of older age on the fisk of myocardial infarction and stroke has been reported in diabetic patients[9]. Independent effects of duration of diabetes and greater risks associated with early rather than late onset of diabetes have been reported[10,11]. In patients with type 2 diabetes, current age, age at diagnosis, and diabetes duration were reported to be independently associated with macrovascular events and death. Diabetes duration was independently associated with microvascular events[9]. Long duration of diabetes, poor glycemic control, and hypertension reportedly increase the chances of microvascular complications of diabetes[11]. Poor glycemic control was associated with

Cite this article : Ali S, Rao N L. **Correlation of serum fluorescence of advanced glycation end products with diabetes duration and glycemic control in type 2 diabetic patients.** *Biomed. Res. Ther.*; 7(8):3933-3938.