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Role of glycated hemoglobin in microvascular complications in type 2 diabetes mellitus: cross sectional study

Abstract. Background. Diabetes mellitus (DM) is a complex disorder which defects mainly vascular complications. Complications increase the morbidity and mortality associated with the disease, reducing life expectancy by 10–15 years. Diabetic neuropathy (DN) is a frequent complication of DM and is defined as the presence of peripheral nerve dysfunction after exclusion of other causes. Poor glycaemic control and chronic hyperglycaemia are the major risk factors for DN. Most important treatment of DN remains good glucose control generally noted as HbA1c ≤ 7.5 %. The purpose of this cross-sectional study is to investigate the role of glycated hemoglobin in microvascular complications in type 2 diabetes mellitus. Materials and methods. This cross-sectional study was carried out in Teerthanker Mahaveer Medical College and Research Center, Moradabad from Jan 2016 to December 2016 in which 100 type 2 diabetic mellitus (T2DM) patients in the age group of 35–69 years were included. Patients were divided into 2 groups of 50 each. Group 1 constituted of T2DM patients without any microvascular complication and group 2 includes T2DM patients with microvascular complications. Examination of patients included recording of medical history, pulse rate and blood pressure. Neuropathy was assessed by clinical examination based on modified NDS procedure, which included examination of vibration, pin prick sensation, temperature sensation on dorsum of foot and Achilles tendon reflex. Blood samples were collected by venepuncture and accordingly biochemistry analysis was carried out (Fasting Blood Glucose, post prandial blood glucose and HbA1c were recorded). HbA1c estimation was done by using COBAS fully automated analyser. Results. Among recruited patients, Mean fasting, PPBS level, HbA1c among test group was significantly more than control group. In control group significant correlation of HbA1c is found with age, weight and BMI and in test group significant correlation of HbA1c is observed with age and duration of DM. Significant correlation of HbA1c with FBS and PPBS seen in both control group and in test group. Among test group, 19 patients were found to have DN and significant correlation of HbA1c is observed with NDS score. Conclusions. Increased HbA1c is closely associated with DN in T2DM patients and could be considered as a potent indicator for DN in these patients.

Keywords: type 2 diabetes mellitus; diabetic neuropathy; glycated haemoglobin; correlation

Introduction
Diabetes mellitus (DM) is a metabolic disorder of multiple aetiology characterised with disturbances of carbohydrate, fats and protein metabolism results from defects in insulin secretion, insulin action, or both. The effects of DM include long term damage; dysfunction and failure of various organs, such as retinopathy with potential blindness; nephropathy that may lead to renal failure and/or neuropathy with risk of foot ulcers; amputation; Charcot joints; and features of autonomic dysfunction. Patients with DM are at an increased risk of cardiovascular, peripheral vascular and cerebrovascular diseases. This definition highlights the complexity of this disorder and that vascular complications are an essential part of this vascular disorder [1].

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Hyperglycaemia tends to develop very gradually, in the early stages, patients are often asymptomatic as the hyperglycaemic levels are not severe enough to produce symptoms. Because of this, condition may remain undiagnosed for many years and patients present with established macrovascular and microvascular complications upon initial diagnosis of type 2 DM (T2DM) [2].

T2DM is a global epidemic with an estimated worldwide prevalence of 6.4% (285 million) in 2010 that is forecast to rise to 7.7% (438 million) in 2030. In addition, 344 million people have impaired glucose tolerance that is forecast to increase to 472 million by 2030 [3]. India has a higher prevalence of DM (4.3%) as compared with the west (1–2%) as probably Asian Indians are more prone for insulin resistance and cardiovascular mortality [4].

The pathogenic mechanism for microvascular complications in diabetes is rooted in chemical reactions between by-products of sugar and proteins that occur over the course of days or weeks and eventually produce irreversible cross linked protein derivatives called advanced glycosylation end products [5]. There is insufficient clarification of the cause of diabetic neuropathy (DN) but ischaemic and metabolic components are implicated. Activation of polyol pathway in the nerve through enzyme aldose reductase leads to accumulation of sorbitol and fructose in the nerve and induces non-enzymatic glycosylation of structural nerve protein [6].

Hyperglycaemia also induces oxidative stress. Activation of protein kinase has been linked to vascular damage in DN. Peripheral nerve damage includes neuronal degeneration and impairment of regeneration of thinly myelinated fibres mediated by AGE accumulation, activation of polyol and PKC pathways and deleterious effects of reactive oxygen species [6].

DN is recognised by the ADA as ‘the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes’ [7]. Long standing hyperglycaemia is the main culprit in the development of DN. This has been shown in the results of DCCT. This randomised prospective study showed significant reduction in the development and progression of clinical neuropathy (64%), motor conduction velocity (44%) and autonomic dysfunction (53%) in type 1 DM with optimal glycaemic control [8].

DN prevalence differs in observational studies due to the varying diagnostic methods used [9]. Screening for DN is of high importance since approximately 50% of patients with DN are asymptomatic [10]. DN increases the risk of lower limb amputations by 1.7-fold [11]. This increased frequency of lower limb amputation in patients with DM and diabetic neuropathy is attributed to lower limb microtraumatism, due to the fact that affected individuals have diminished pain sensation.

Most investigated and documented predictor factors for the development of DN are hyperglycaemia, DM duration and age as well as the presence of microvascular complications including hypertension, dyslipidaemia, diabetic retinopathy and chronic kidney disease [12].

The varied clinical presentations and manifestations of neuropathy among diabetic patients reflect the heterogeneity of this problem. Peripheral neuropathy is a common diabetic complication and like retinopathy and nephropathy is related to age, duration of diabetes and glycaemic status of the patients. D.J. Fernando et al. demonstrated that mean vibration perception threshold and nerve conduction velocity are abnormal in patients with microalbuminuria and in those with overt nephropathy [13]. Study on 440 diabetic patients who were followed up over 25 years, showed an increase in clinically detectable DN from 12% at the time of diagnosis of diabetes to about 50% after 25 years and those with poorest diabetes control had highest prevalence [14]. In UKPDS, control of blood glucose was associated with improvement in vibration perception [15].

Glycated hemoglobin (HbA1c) level was seen as the most important factor predicting higher risk of subclinical neuropathy, therefore nerve conduction studies abnormality commonly exists in diabetic patients in the subclinical stages of polyneuropathy and are highly correlated to HbA1c levels. Prevention and early detection of DN assumes an utmost importance as two-third of patients have clinical or sub clinical neuropathy at the time of diagnosis NDS has been widely accepted and validated tool to identify the presence of DN. Electrodiagnostic examination being a sophisticated examination and not available in hospitals with low resource settings hence, to study the correlation of DN with HbA1c on basis of NDS scoring system is much needed.

The purpose of this cross-sectional study is to investigate the role of glycated hemoglobin in microvascular complications in type 2 diabetes mellitus.

Materials and methods

This prospective cross-sectional (hospital based) study was carried in TMMC and Research centre after approval from Institutional Ethics Committee. Data for the study was collected for a period of 12 months and included patients of T2DM who attended the diabetes clinic from the period January 2016 — July 2016 after obtaining written informed consent.

Study Population:

Study included 100 T2DM patients and divided into 2 groups of 50 each. Group 1 constituted of 50 DM patients without any complication and group 2 consisted of another 50 with microvascular complication for one year.

Inclusion criteria:

1. Patients suffering from T2DM.
2. Patients in the age group 35–69 years of both sexes.
3. Patients who are willing to give informed consent.

Exclusion criteria:

1. No previous systemic condition related to peripheral neuropathy.
2. Any neuromuscular diagnosis such as myopathy, familial polyneuropathy, chronic polyneuropathy.
3. Neuropathy associated with exogenous toxins, metals or drugs.
4. Pregnancy and post-menopausal with HRT.

Data collection:

Basic general information of study population was recorded and medical history included: duration of DM, past medical history, family history, smoking status, alcohol intake and drug history.

Patients underwent battery of investigations including FPG, PPBG, and HbA1c. Blood samples were collected by venepuncture after ensuring 8 hours of overnight fasting. Blood samples were taken in EDTA disodium coated
and plain vials and centrifuged to obtain plasma and serum and accordingly biochemistry analysis was carried out. Biochemical investigations included HbA1c (done using Cobas HbA1c test), fasting blood glucose and post prandial blood glucose (GOD-POD method).

For peripheral neuropathy:

Sensory examination was done in a quiet and relaxed setting. Neuropathic deficits in the feet were determined using the NDS, derived from the examination of Vibration (using a 128 Hz tuning fork), pin prick sensation (apply pin proximal to big toe nail just enough to deform the skin), temperature perception on dorsum of foot (using warm and cold water in test tubes) and Achilles tendon Reflex (using a tendon hammer).

The 3 perceptions were scored 0 if present and normal; and 1 if absent, reduced, or uncertain. On either side, the ankle reflex was scored 0 if present and normal and 2 if absent the maximum deficit score is 10 which would indicate complete loss of sensation to all sensory modalities and absent reflexes. A score of 6 or more has been found to indicate an increased risk of foot ulceration. Score of 3 or more is defined as positive for peripheral neuropathy.

Statistics

Data was tabulated as Mean ± SD. Results were analysed using non-parametric test (Chisquare and Mann-Whitney U test) and parametric test (t-test). In addition, correlation between 2 numerical variables was done using Pearson correlation coefficient analysis. P value of < 0.05 was considered statistically significant.

Results

In this study 100 type 2 diabetic patients were examined out of which 50 patients constituted the control group (diabetic patients without microvascular complications) and 50 constituted the test group (diabetic patients with any microvascular complication).

In the control group 68 % patients were male and 32 % patients were females. In the test group 64 % patients were male and 36 % patients were females. Mean fasting and post prandial blood sugar level among test group (291.02 ± 38.04 and 322.46 ± 40.12 mg/100 ml) was significantly higher than control group (187.9 ± 36.49 and 219.34 ± 38.86 mg/100 ml respectively). Mean HbA1c among test group (11.68 ± 1.29 %) was significantly higher than control group (8.26 ± 1.09 %) (table 1).

Among the test group; 48 patients were affected with DN, 19 were affected with neuropathy and 34 were affected with retinopathy. Among the test group; significant correlation of HbA1c was observed with NDS score (r = 0.640). Table 2 severity of hyperglycaemia and abnormal HbA1c levels considerably affects the result of NDS testing.

Discussion

Practically every system is affected by complications of DM. Microvascular complications are the major outcome of T2DM progression, reduces the quality of life and increases diabetic mortality. Because of insidious and silent onset of T2DM, this disease acts as a silent killer. Large proportion of T2DM patients may have elevated blood sugar levels for several years prior to diagnosis and present with various microvascular complications at the time of diagnosis.

HbA1c is a minor red cell constituent that comprises 5 % of the total Hb in normal individuals but up to 15 % in patients with DM. HbA1c levels are a reflection of patient’s average blood sugar for preceding 2–3 months and this test is not affected by recent physical activity and/or emotional fluctuations.

In present study there is increased incidence of T2DM in males as compared to females in both test and the control group. Previous studies show that male gender is the risk factor for the development of anyone of the microvascular complications. A study conducted in T2DM subjects by A.H. Alrawahi et al. in Oman has also shown positive relationship with male gender [16].

Study of Y.C. Yang et al. showed increased HbA1c level in males. It seems to be related to the lower Hb level in menstruating females with more rapid erythrocyte turn over [17].

FBS, PPBS, HbA1c levels are higher in test group than in control group. DM with elevated blood glucose levels for long period causes increased and rapid non-enzymatic glycation of collagen and elastic fibres. This reflects that increased HbA1c level is an indicator of assessing the severity of microangiopathies in diabetic patients.

Diabetic patients with higher HbA1c level are more prone to develop microangiopathies. In our study, 36 % diabetic patients had one microvascular complication at the time of diagnosis, 34 % had 2 complications and 30 % had all 3 complications.

Study of R. Raman et al. showed that nearly one-third of the newly diagnosed type 2 diabetic subjects had some form of microvascular complication; nephropathy and neuropathy being more common than retinopathy [18].

### Table 1. Biochemical characteristics of patients in both groups (Mean ± SD)

| Parameter                      | Control Group (n = 50)               | Test Group (n = 50)               |
|--------------------------------|-------------------------------------|----------------------------------|
| Fasting blood glucose (FBS), mg/100 ml | 187.90 ± 36.49                      | 291.02 ± 38.04*                  |
| Post prandial blood glucose (PPBS), mg/100 ml | 219.34 ± 38.86                      | 322.46 ± 40.12*                  |
| Glycated hemoglobin (HbA1c), %     | 8.26 ± 1.09                         | 11.68 ± 1.29*                    |

Notes: Unpaired ‘t’-test Mann-Whitney U Test; * — significant difference.

### Table 2. Correlation between neuropathy score and HbA1c in test group

| Variables | r value | p value |
|-----------|---------|---------|
| NDS Score | 0.64    | < 0.05* |

Note: *p < 0.05 using Pearson correlation coefficients.
In our study, incidence of DN is the least among all the microvascular complications and some correlation exists between diabetic nephropathy and retinopathy. Microvascular complications are the major outcome of T2DM progression which reduces the quality of life and increases diabetic mortality. Study of H.K. Kumar et al. showed that incidence of neuropathy 3 %, nephropathy 20 % and retinopathy 48 % of study population [19]. Type 2 diabetic patients with poor glycaemic control should be strongly encouraged for optimal or even good tight glycaemic control in order to prevent early emergence of microvascular complications. Screening with simple tests is essential to identify the complication at an early reversible stage.

In the control group significant correlation of HbA1c level has been observed with age, weight and BMI. HbA1c shows significant correlation with FBS and PPBS. Significant correlation between FBS, PPBS and RBS with HbA1c is seen in various studies, although PPBS showed marginally better correlation in comparison to FBG.

In the test group, significant correlation of HbA1c is seen with age and duration of DM. With advancement in age and duration of diabetes there is gradual tendency for the level of blood sugar to rise along with subsequent increase in HbA1c. Amount of carbohydrate attached to HbA1c increases with increasing duration of diabetes.

Significant correlation of HbA1c with neuropathy has been observed in the study which shows that HbA1c is an important indicator for predicting the neuropathies. Study of K. El-Salem et al. reported HbA1c to be the most important predicting factor for higher risk of subclinical neuropathy. Nerve conduction abnormalities commonly exist in diabetic patients in the subclinical stages and are highly correlated to HbA1c levels [20].

Study of R.B. Paisey et al. showed that tall stature and worse metabolic control were associated with progression to neuropathy. Mean HbA1c levels were higher in those who develop foot ulcers. This is consistent with the hypothesis that the severity of the metabolic disturbance in DM influences susceptibility to neuropathy. There is a possibility that intense reduction in glycaemia from diagnosis could improve peripheral nerve function [21].

Conclusions

Glycaemic status of DM patients is an important risk factor for development of microvascular complications and patients’ profile having microvascular complications shows that they have poor glycaemic control. The duration of DM had a positive correlation with incidence of microangiopathy. Thus, DM patients with microvascular complications had longer duration of DM as compared to diabetics without microangiopathy.

The role of poor glycaemic control and chronic hyperglycaemia are the major risk factors for diabetic neuropathy. The severity of hyperglycaemia and abnormal glycated hemoglobin levels considerably affects the results of the NDS testing. Timely screening and earlier detection and intervention are useful in preventing the progression of neuropathy and reduced risk of complications in other organs. It is recommended that a target HbA1c remain as close to normal as possible, which will provide improved outcome. Tight glycaemic control, which is measured by HbA1c is the most important factor to decrease the microvascular events.

Since HbA1c level is proportional to an average blood glucose concentration over the previous 4 weeks to 3 months and is not influenced by recent physical and/or emotional fluctuation, it gives an idea to follow proper routine.

References

1. American Diabetes Association Professional Practice Committee. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2022. Diabetes Care. 2022 Jan 1;45(Suppl 1):S17-S38. doi:10.2377/dc22-S002.

2. Westman EC. Type 2 Diabetes Mellitus: A Pathophysiological Perspective. Front Nutr. 2021 Aug 10;8:707371. doi:10.3389/fnut.2021.707371.

3. Khan MAB, Hashim MJ, King JK, Gounder RD, Mustafa H, Al Kaabi J. Epidemiology of Type 2 Diabetes - Global Burden of Disease and Forecasted Trends. J Epidemiol Glob Health. 2020 Mar;10(1):107-111. doi:10.2991/jeigh.k.191028.001.

4. Mathur P, Leburu S, Kulothungan V. Awareness, Treatment and Control of Diabetes in India From the Country-Wide National NCD Monitoring Survey. Front Public Health. 2022 Mar 14;10:748157. doi:10.3389/fpubh.2022.748157.

5. Faselis C, Katsimardou A, Impriolos K, Deligkaris P, Kallistratos M, Dimitriadis K. Microvascular Complications of Type 2 Diabetes Mellitus. Curr Vasc Pharmacol. 2020;18(2):117-124. doi:10.2174/1570161117666190502103733.

6. Sloan G, Selvarajah D, Teufsey S. Pathogenesis, diagnosis and clinical management of diabetic sensorimotor peripheral neuropathy. Nat Rev Endocrinol. 2021 Jul;17(7):400-420. doi:10.1038/s41574-021-00496-z.

7. Pop-Busui R, Boulton AJ, Feldman EL, et al. Diabetic Neuropathy: A Position Statement by the American Diabetes Association. Diabetes Care. 2017 Jan;40(1):136–154. doi:10.2337/dc16-2042.

8. Nathan DM, Genuith S, Lachin J, et al. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med. 1993 Sep 30;329(14):977-986. doi:10.1056/NEJM199309303291401.

9. Shiferaw WS, Akalu TY, Work Y, Aynalem Y. Prevalence of diabetic peripheral neuropathy in Africa: a systematic review and meta-analysis. BMC Endocr Disord. 2020 Apr 15;20(1):49. doi:10.1186/s12902-020-00334-5.

10. Sun J, Wang Y, Zhang X, Zhu S, He H. Prevalence of peripheral neuropathy in patients with diabetes: A systematic review and meta-analysis. Prim Care Diabetes. 2020 Oct;14(5):435-444. doi:10.1016/j.pcd.2019.12.005.

11. Alothman S, Alencuazi A, Waitsman LR, LeMaster J, Kluding P. Neuropathy and Other Risk Factors for Lower Extremity Amputation in People with Diabetes Using a Clinical Data Repository System. J Allied Health. 2018 Fall;47(3):217-221.

12. Liu X, Yu Y, An M, Zeng Q. The risk factors for diabetic peripheral neuropathy: A meta-analysis. PLoS One. 2019 Feb 20;14(2):e0212574. doi:10.1371/journal.pone.0212574.

13. Fernando DJ, Hutchinson A, Yeves A, Gokal R, Boulton AJ. Risk factors for non-ischaemic foot ulceration in diabetic nephropathy. Diabet Med. 1991 Apr;8(3):223-225. doi:10.1111/j.1464-5491.1991.tb01576.x.

14. Lu Y, Xing P, Cai X, et al. Prevalence and Risk Factors for
Цукровий діабет (ЦД) — складний клінічний процес, який виникає з тривалого надмірного вмісту глюкози в організмі через підвищення продукції і розсміщення глюкози із крові. Недіагностований життєвий ситуації, ЦД може викликати ряд гострих та хронічних порушень, включаючи нефропатію, нервову патологію та дисплазію. Нейропатія, артеріальний тиск та задоволення споживання продуктів включають вивчення анамнезу, частоти серцевих скорочень. Обстеження пацієнтів проводили за допомогою автоматизованого аналізатора COBAS. Оцінку HbA1c проводили з використанням тестів, включаючи аналізатор COBAS.

Резюме. Актуальність. Цукровий діабет (ЦД) — складний розлад, що супроводжується переважно судинними ускладненнями. У майбутньому, з розвитком глюкометрії та інших тестів, можлива адекватна діагностика та лабораторна оцінка глюкози та глюкози в крові натоще і постпрандіально.

Гілкований гемоглобін (HbA1c) є клінічним показником нейропатії. Нейропатію оцінюють шляхом вивчення ролі глікованого гемоглобіну в мікросудинних ускладненнях. У майбутньому, з розвитком глюкометрії та інших тестів, можлива адекватна діагностика та лабораторна оцінка глюкози та глюкози в крові натоще і постпрандіально.

Результати. У контрольній групі були вірогідно вищими, ніж у контрольній групі. У контрольній групі були вірогідно вищими, ніж у контрольній групі. У контрольній групі були вірогідно вищими, ніж у контрольній групі.