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Application of multiple linear regression and machine learning algorithms to elucidate the association of poor glycemic control and hyperhomocysteinemia with microalbuminuria

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Microalbuminuria is an early biomarker of general vascular dysfunction and a predictor of risk for cardiovascular and renal diseases. It is also considered as a marker of insulin resistance in both diabetic and non-diabetic patients. The rationale of this study was to elucidate threshold values of fasting blood glucose (FBS) and glycosylated hemoglobin (HbA1c) that are associated with microalbuminuria. In the parallel association of microalbuminuria with hyperhomocysteinemia was investigated. Machine learning algorithm and multiple linear regression were applied to study the association of poor glycemic control on microalbuminuria and hyperhomocysteinemia. In non-diabetic subjects with FBS <102 mg/dL and HbA1c <6.3%; and in diabetic subjects with good glycemic control (FBS: 102-118 mg/dL; HbA1c: 6.3-7.0%), urinary microalbumin levels were <40µg/mg creatinine. Poor glycemic control (FBS >172 mg/dL and HbA1c >9.0%) was associated with microalbumin >40µg/mg creatinine. Age, gender, HbA1c and FBS were shown to explain variability in urinary microalbumin to the extent of 54.4% as shown by multiple linear regression model. Analysis of variance (ANOVA) revealed higher levels of FBS (F: 39.77, *P* <0.0001), HbA1c (F: 64.31, *P* <0.0001) and total plasma homocysteine (F: 3.69, *P* =0.04) in microalbuminuria and clinical microalbuminuria groups when compared to subjects with normal microalbumin levels. Diabetic patients with poor glycemic index had a more B_{12} deficiency. Poor glycemic index and hyperhomocysteinemia were associated with clinical microalbuminuria.

Keywords: Diabetes, HbA1c, Homocysteine, Machine learning algorithms, Microalbuminuria

India is emerging as the diabetes capital of the world with 62 million individuals affected with diabetes[1]. The global prevalence of microalbuminuria was reported to be 39%[2]. The duration of diabetes and HbA1c levels were reported to have positive association with urinary microalbumin levels[3,4]. Urinary proteome analysis of microalbuminuria cases revealed α2-HS-glycoprotein, vitamin D binding protein, CD59, an extracellular matrix protein 1 (ECM1), factor H and myoglobin[5]. The α2-HS-glycoprotein was associated with functions such as endocytosis, brain development and formation of bone tissue. Vitamin D binding protein binds vitamin D2, vitamin D3 and their 25-hydroxylated forms and facilitates their transport across different tissues. CD59 causes microvesicular damage while ECM1 and factor H influence mesangial cell proliferation. 25-hydroxy vitamin D deficiency was reported to be associated with diabetes and renal disease[6].

A recent study showed elevated plasma homocysteine levels in type II diabetes mellitus and this elevation increases further due to increased insulin resistance and poor glycemic control[7]. In healthy adults, plasma homocysteine was reported to be positively associated with microalbumin in men[8]. Patients with type II diabetes, long-term treatment with metformin and smoking were shown to have a higher prevalence of vitamin B_{12} deficiency[9]. B_{12} supplementation in children was shown to influence the regulation of several metabolically important type II diabetes-associated genes through methylation of miR 21[10].

In view of the positive association of diabetes and HbA1c with microalbuminuria, the association of treatment-induced vitamin B_{12} deficiency in type II diabetes, the current study was planned to investigate the thresholds of fasting blood glucose, HbA1c

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and total plasma homocysteine associated with microalbuminuria and clinical microalbuminuria.

Materials and Methods

Recruitment of subjects

We have enrolled a total of 975 subjects, which includes 873 diabetic patients (514 men and 359 women) and 102 non-diabetic controls (57 men and 45 women) at the out-patient unit of Nizam's Institute of Medical Sciences, Hyderabad, India. The mean ages in diabetic and non-diabetic patients were 60.6±13.1 year and 58.9±10.5, respectively. The study protocol was approved by the Institutional Ethical committee of Nizam's Institute of Medical Sciences, Hyderabad, India. All the enrolled subjects consented for the study.

Biochemical evaluation

Blood glucose levels were estimated by hexokinase method using Roche Cobas c501 fully automated chemistry analyzer (Roche Diagnostics, U.S.A.). HbA1c levels were estimated by using the Bio-Rad D-10TM Dual Program intended for the percent determination of HbA1c in human whole blood using ion-exchange high-performance liquid chromatography (HPLC). The samples are automatically diluted on the D-10 and injected into the analytical cartridge. The D-10 delivers a programmed buffer gradient of increasing ionic strength to the cartridge, where the hemoglobins are separated based on their ionic interactions with the cartridge material. The separated hemoglobins then pass through the flow cell of the filter photometer, where changes in the absorbance 415 nm are measured. The A1c area is calculated using an exponentially modified Gaussian (EMG) algorithm that excludes the labile A1c and carbamylated peak area from the A1c peak area. Immunoturbidimetric assay on Roche Coas c501 analyzer was used to estimate urinary microalbumin levels. Anti-albumin antibodies react with the antigen in the sample to form antigen/antibody complexes which, following agglutination, are measured turbidimetrically. Vitamin B_{12}, Folate and Homocysteine assays are performed on ADVIA Centaur® XP Immunoassay system using kits manufactured by Simens Healthcare Diagnostics Inc., U.S.A.

Statistical analysis

Student *t*-test was performed for bivariate and ANOVA was performed for trivariate datasets of continuous variables. Tetraplot software was used to plot trivariate models. The multiple linear regression model was developed using age, gender, FBS, HbA1c and diabetic status as the predictors and urinary microalbumin levels as the output using www.wessa.net. The association statistics were based on a machine learning algorithm developed using the computational software "www.bigml.com".

Results

The diabetic subjects had elevated microalbumin levels in comparison to non-diabetic subjects (79.0±185 *vs.* 3.51±1.55 µg/mg creatinine, P <0.0001). The FBS levels in non-diabetic subjects were 97.17 ± 13.7 mg/dL. The FBS levels in diabetic patients were segregated based on HbA1c levels as shown below: HbA1c<6.0%: 112.29 ± 26.9 mg/dL; HbA1c: 6.0-8.0 %: 125.30 ± 29.7 mg/dL; HbA1c >8.0-10.0%: 161.57 ± 51.9 mg/dL; and HbA1c >10.0%: 234.29 ± 81.3 mg/dL. Analysis of variance (ANOVA) revealed that FBS levels are associated with glycosylated hemoglobin (F: 187.3, P <0.0001).

The microalbumin data was segregated into normal microalbumin (<30 µg/mg creatinine), microalbuminuria (30-300 µg/mg creatinine) and clinical microalbuminuria (>300 µg/mg creatinine). Further, segregation was done based on glycosylated hemoglobin. All cases of microalbuminuria were shown to have glycosylated hemoglobin >6.0 except one. The prevalence of microalbuminuria in subjects with HbA1C levels 6.0-8.0%, >8.0-10.0% and >10% were 26.3%, 33.7% and 66.0%, respectively, (χ2: 87.98, P <0.0001) while the prevalence of clinical microalbuminuria were 5.9%, 10.1% and 29.8%, respectively, (χ2: 14.54, P =0.0001).

The fasting blood glucose levels were within the renal threshold range in normal microalbumin group *i.e.*131.69 ±43.9 mg/dL. In microalbuminuria and clinical microalbuminuria the FBS levels are 163.73 ± 70.0 mg/dL and 171.79 ± 86.9 mg/dL, respectively, (F: 39.77, P <0.0001). Trivariate plot (Fig. 1) clearly demonstrates that in subjects with HbA1c <6.0% and fasting blood glucose within the renal threshold limit, there was no association with microalbuminuria.

As illustrates in (Fig. 2), B_{12} deficiency was associated with poor glycemic index while folate status has no association. Clinical microalbuminuria was shown to be associated with HbA1c >8% and homocysteine >32 µM/L (Fig. 3).

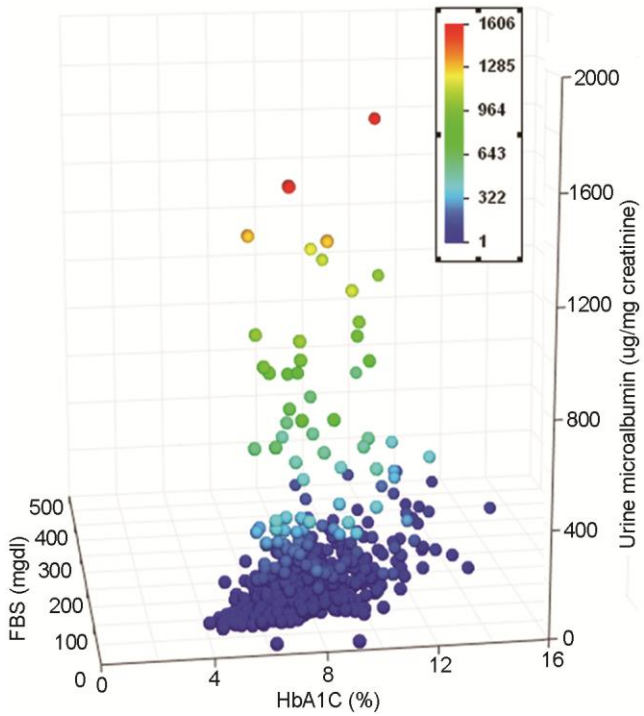

Fig. 1 — Association of Glycosylated hemoglobin and Fasting blood sugar with urinary microalbumin. This clearly illustrates that Glycosylated hemoglobin <6.0% and Fasting blood sugar <100 mg/dL is not associated with microalbuminuria

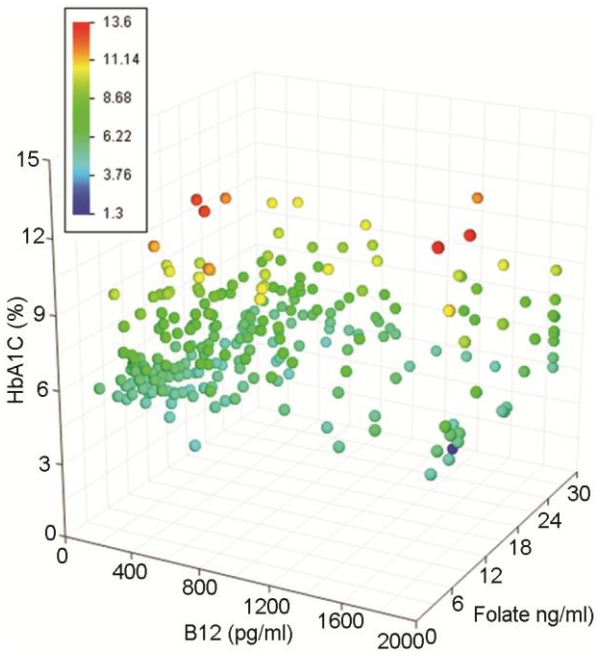

Fig. 2 — Association of B_{12} and folate with glycemic index. This illustrates that low B_{12} status is associated with the poor glycemic index while folate status has no influence on the glycemic index

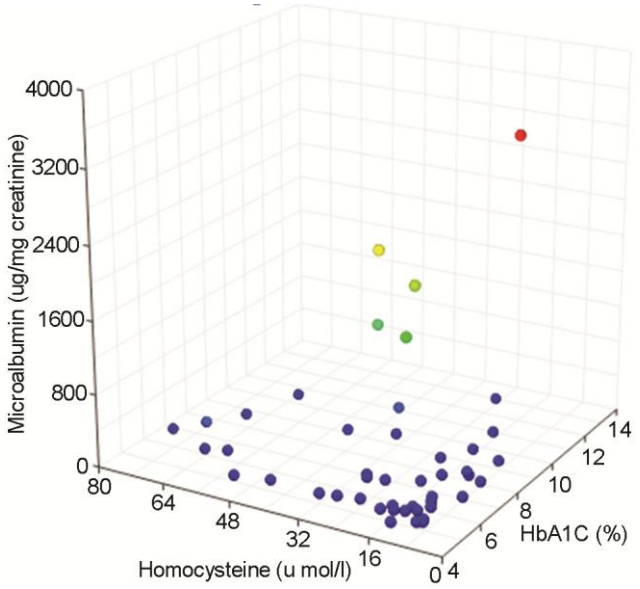

Fig. 3 — Association of homocysteine and HbA1c with microalbuminuria. This illustrates that clinical microalbuminuria is associated with poor glycemic index (HbA1c > 8%) and elevated homocysteine (>32 uM/L)

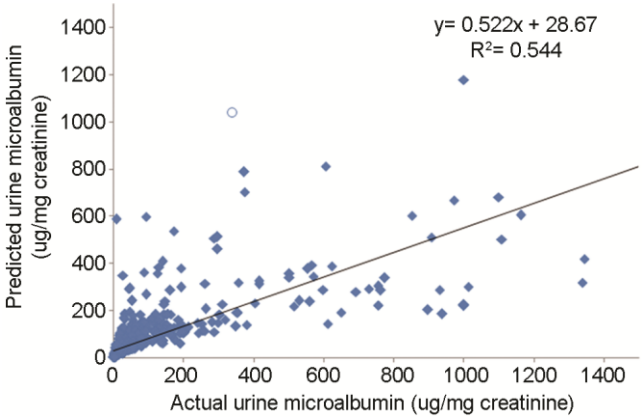

Fig. 4 — Correlation of experimental microalbumin levels with predictions based on multiple linear regression. The experimental microalbumin levels were correlated with multiple linear regression based predicted values (r^2: 0.544)

Furthermore, we have deduced the following equation to depict the contribution of different variables towards urinary microalbumin using the multiple linear regression model:

Log 10 (Urine microalbumin) = −0.237477 + [0.00288253×age] + [0.0442243×gender] + [0.0811325×glycosylated hemoglobin] + [0.000990878×Fasting blood sugar] + [0.589106×diabetic]

This model explained 54.4% variability in urinary microalbumin (Fig. 4). The association statistics

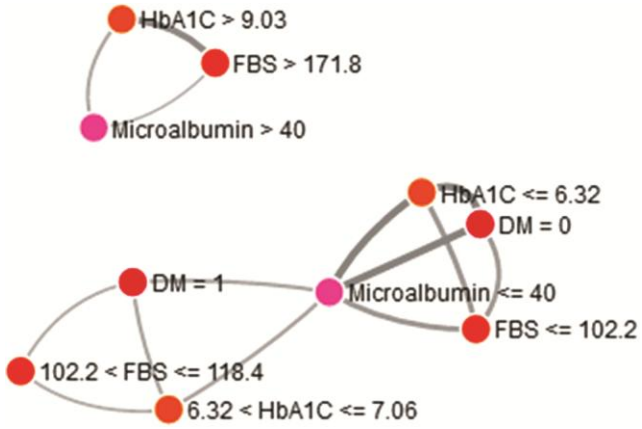

Fig. 5 — Association statistics of microalbuminuria based on a machine learning algorithm. The association statistics reveal that non-diabetic subjects with FBS <102 mg/dL and Hb1Ac <6.32; and diabetic subjects with FBS in the range of 102-118 mg/dL and HbA1c 6.32-7.06% will have microalbumin levels <40 µg/mg creatinine. Subjects with uncontrolled diabetes *i.e.* FBS >171.8 and HbA1C >9.03% are associated with microalbumin >40 µg/mg creatinine

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Discussion

The current study adds a new dimension to the already documented association of poor glycemic index and microalbuminuria by providing the thresholds of fasting blood glucose and HbA1c that are strongly associated with microalbuminuria. In order to establish these thresholds, machine learning algorithms were used for the first time. FBS >172 mg/dL and HbA1c >9.0 are associated with urine microalbumin >40 µg/mg creatinine. This is in agreement with another Indian study that demonstrated an increase in microalbumin levels with an increase in HbA1c[11].

The renal threshold of glucose in type II diabetes patients was reported to vary based on age, disease duration and body mass index[12]. A large-scale study demonstrated that urinary albumin-creatinine ratio, age, weight, HbA1c, blood glucose, total cholesterol, antihypertensive drugs and heart rate as the key determinants of microalbuminuria in diabetes patient[13], which is in agreement with our multiple linear regression model. Another large-scale study applied multiple logistic regression model and demonstrated that microalbuminuria was associated with hyperglycemia and HbA1c in the non-obese group; and hypertension and HbA1c in the obese group in younger subjects[14]. Even in prediabetic subjects who have a genetic predisposition to diabetes due to Glucokinase (GCK) rs2908289, fasting blood glucose was associated with microalbuminuria even after adjusting for confounding factors such as age, gender, smoking, systolic blood pressure, waist circumference and serum triglycerides[15]. Prediabetic subjects were reported to have interleukin-6 (IL-6) and hyperinsulinemia indicating subclinical inflammation and insulin resistance even in prediabetic period[16]. Strong positive association was observed between IL-6 and insulin in these subjects[16].

Consistent with our study, total plasma homocysteine levels were found to be higher in diabetic subjects and were positively associated with microalbuminuria[17]. Higher incidence of B_{12} deficiency was reported in the elderly population, with longer diabetes mellitus duration, hypothyroidism and treatment with metformin[18]. Our results are in agreement with Passaro *et al.*, in demonstrating association of poor glycemic control with hyperhomocysteinemia[19]. Subjects with microalbuminuria were reported to have higher blood pressure and homocysteine levels compared to those with normal microalbumin levels[20]. Dietary glycemic index was shown to have a positive association with homocysteine[21]. In type II diabetes patients, renal mishandling of thiamine increased degradation of vitamin B_6 and cytosolic metabolic resistance to vitamin B_{12} was reported[22]. A recent meta-analysis reported normal homocysteine levels in type I DM patients without any complications, however, in subjects with T1DM associated microvascular complications, such as diabetic retinopathy and diabetic neuropathy, hyperhomocysteinemia was observed[23]. In subjects with type II diabetes, the baseline plasma homocysteine and mean HbA1c levels were reported to be independent predictors of microalbuminuria[24].

The strength of the current study are: (i) large sample size; (ii) application of multiple linear regression to understand the contribution of different variables towards microalbuminuria; (iii) establishment of thresholds of fasting blood glucose and HbA1c that contribute to microalbuminuria. The limitations are: (i) diabetes subjects were not grouped into type I or type II diabetes; (ii) information on diet

and other lifestyle risk factors were not available. Future studies are warranted to investigate whether type I and type II diabetes subjects behave differently towards microalbuminuria.

Conclusion

Subjects with FBS >172 mg/dL and HbA1c >9.0% are associated with urine microalbumin >40 µg/mg creatinine. B_{12} deficiency was observed in subjects with poor glycemic index. Clinical microalbuminuria is associated with hyperhomocysteinemia (>32 µM/L).

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