

Comparison of serum folic acid and Vitamin B12 among type 2 diabetes mellitus patients with and without nephropathy; a cross sectional observational study from eastern India

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Abstract

Background & aims: Diabetic nephropathy is the leading cause of chronic kidney disease and end-stage renal disease. According to earlier studies serum folic acid and vitamin B12 were found to be reduced in such patients. Therefore, the present study was conducted to estimate and compare the serum folic acid and vitamin B12 values of diabetic mellitus patients with and without nephropathy. **Materials and methods:** A single institution based cross sectional study was conducted in Kolkata from March 2018 to September 2019. 203 adult with at least five years history of type 2 diabetes mellitus were subdivided into two groups' nephropathy and non-nephropathy. The cut off value for inclusion into nephropathy group was urinary albumin-creatinine ratio of more than equal to 30gm/dl. Serum folic acid and vitamin B12 values of both groups were estimated by Chemiluminescence assay and later statistically compared for result. **Result:** Overall 127 patients were grouped in non-nephropathy while rest 76 belonged to nephropathy. Both folic acid (4.68 ± 3.64 versus 7.02 ± 3.89 ; $p < 0.0001$) and Vitamin B 12 (219.54 ± 56.53 versus 343.98 ± 32.1 ; $p < 0.0001$) values were found to significantly lower in the nephropathy group. Non-nephropathy patients also displayed better glycemic profile in terms of HbA1c. **Conclusion:** Patients with diabetic nephropathy displayed lower levels of serum folic acid as well as vitamin B12 in comparison to the patients who did not develop nephropathy. Besides renal parameters the glycemic statuses of nephropathy patients were also poor.

Keywords: Diabetic nephropathy, folic acid, Vitamin B12, urinary albumin –creatinine ratio, Chemiluminescence assay.

Introduction

Diabetic nephropathy (DN) which is also referred as Diabetic Kidney Disease (DKD) is the leading cause of Chronic Kidney disease (CKD) and End-stage renal disease (ESRD) [1]. It is recognized as one of the macro vascular complication of Diabetes mellitus (DM) and develops in 40% of patients with type 2 DM and 30% with type 1 DM [2]. Clinically DKD is characterized by persistent albuminuria, hypertension and a progressive fall of glomerular filtration rate (GFR).

Several biochemical and metabolic also changes take place during the progression of DN. Two earlier clinical trials conducted in late 90's have shown the association of low serum folate levels among patients suffering from Diabetes mellitus as well as DN [3, 4]. A similar analysis from New Delhi was also reported in the year 2016 [5].

Besides folic acid, Vitamin B12 deficiency was also linked to the development of the chronic complication of Diabetes mellitus such as DN in previous literature [6]. Lower levels of Vitamin B12 were observed among the DN patients in another study conducted in North India [7].

However, no such analysis was conducted in the eastern part of the country in recent time. Therefore, the current study was undertaken to find out the association of serum folic acid and Vitamin B12 levels among the patients suffering from DN.

Materials and Methods

It was a single institution-based cross-sectional observational study which took place from 1st March 2018 to 30th September 2019. The study was conducted only after obtaining the permission from the Institutional Ethics Committee (MC/KOL/IEC/Non-spon/23/02-2018; dated 3rd February 2018). Altogether 203 adult patients aged between 28 to 77 years with a history of type 2 DM for at least five years; who attended the outpatient departments of Medicine and Endocrinology during the aforesaid period were included for the present study.

A convenience sampling method was adopted while selection of the cases. Patients who were having symptoms of any acute illness or had history of proteinuria due to any other cause were spared from the study. Those who did not wish to provide their blood samples were also spared. Pregnant and lactating mothers were not included either.

After collecting the detailed demographic features of all the participants, through clinical examination was performed. A standardized weighing machine was deployed to calculate the weight in kilograms; whereas a fixed wall mounted height measuring scale was used to find out the heights in meters. Body mass index (BMI) was calculated by the formula kg/m^2 , where kg represented patient's weight in kilograms, whereas m^2 meant height in meter squared.

Later, venous blood samples were collected from each of them by using hypodermic needle under a strict aseptic condition and were sent to the biochemistry department of the same institute for estimation of serum Folic acid, Vitamin B12, spot urinary albumin creatinine ratio (UACR), HbA1C, urea, creatinine, and uric acid levels. For the estimation of serum Folic acid and Vitamin B12 Chemiluminescence assay was done. Normal reference ranges of folic acid and Vitamin B12 were considered 3-20 ng/dl [8] and 200-835pg/dl [8] respectively.

Once the biochemical values were available from the laboratory they were meticulously compiled in a specifically predesigned case report form alongside the other relevant clinical details of the participants. First the entire study population was broadly divided into two groups; namely nephropathy and non-nephropathy on the basis of UACR value. Those who exhibited a value of less than 30mg/gm were included in non-nephropathy group while the nephropathy group consisted of the patients in whom UACR value were more than equal to 30mg/gm. Then the biochemical and clinical details of the two groups were statistically compared for result. Continuous variables were expressed in terms of mean \pm standard deviation (SD). Student's *t*-test was performed to determine the significance of differences between the means of two independent samples. Fischer's exact test was used for the comparison of categorical variables. $P \leq 0.05$ was considered statistically significant. Graph Pad Prism (Developed by Graph Pad Software, Inc; San Diego; California, USA) was employed for the analysis.

Results

After the end of the observation period the data of total 203 patients was analysed. 127 patients who displayed UACR value of less than 30mg/gm were allotted to the non-nephropathy group. On the other hand 76 patients belonged to the nephropathy group since their UACR values were more than equal to 30mg/gm.

The baseline details of the groups were tabulated in table1. No statistically significant differences were observed between in groups in terms of age, male/female ratio and duration of diabetes. The overall duration of diabetes was nevertheless numerically higher in the nephropathy category. Patients from the nephropathy group however had a significantly higher BMI.

Table1: Demographic details of both groups

Parameter	Non-Nephropathy (n=127)	Nephropathy(n=76)	P value
Age in years	60.21± 12.31	62.77±9.03	0.116
Male/ Female (%)	67/60 (52.75/47.25)	43/33 (56.58/43.42)	0.663
BMI	26.74±3.82	28.01±4.19	0.028
History of DM2 (Years)	6.87± 8.79	9.32 ± 10.31	0.061

In table 2 the detailed biochemical parameters of the groups were compared. All the parameters were found to be on the higher side in the nephropathy group. Highly significant statistical differences were detected in UCAR, serum creatinine and serum urea levels based on the available data.

Table 2: Comparison of biochemical details of both groups.

Parameter	Non-Nephropathy(n=127)	Nephropathy(n=76)	P value
UACR (mg/gm)	20.9 ± 10.57	108.16 ± 31.54	<0.0001
Creatinine (mg/dl)	0.91 ± 0.73	1.58 ± 0.89	<0.0001
Urea (mg/dl)	19.18 ± 14.76	49.56 ± 17.87	<0.0001
Uric acid (mg/dl)	4.19 ± 2.07	5.02 ± 2.87	0.018
HbA1c	7.96 ± 1.19	8.42 ± 1.53	0.017

Vitamin B12 and folic acid levels of both groups were statistically compared and expressed in table3. Patients from the nephropathy group in this study showed lower values of both parameters and the differences turned out to be highly significant.

Table 3: Comparison of Vitamin B12 and Folic acid levels between the groups.

Parameter	Non-Nephropathy (n=127)	Nephropathy (n=76)	P value
Serum folic acid (ng/dl)	7.02 ± 3.89	4.68 ± 3.64	<0.0001
Serum B12 (pg/dl)	343.98 ± 32.1	219.54 ± 56.53	<0.0001

Discussion

Diabetic nephropathy is one of the major complications of DM2 and it causes alteration of various biochemical parameters gradually with the progression of the disease. On the basis of previous literatures the present study focused on two such metabolic parameters; serum folic acid and Vitamin B 12 among the patients suffering from diabetic nephropathy from a hospital based patient population.

There were non-significant differences in almost all demographic parameters between the two groups in the current analysis which closely resembled with two previous studies ^[5, 7]. Significantly raised BMI in the nephropathy category was due to fluid accumulation as a result of impaired renal function.

All the renal parameters including serum urea, creatinine, UCAR and uric acid were found to be significantly lower among the patients allotted to the category of non-nephropathy. These findings were also in total concurrence with the earlier literatures reported by Bherwani et al ^[5, 7].

Besides renal profile, the present study also compared the glycemic profile of the patients from both groups unlike previous reports ^[5, 7]. The glycated haemoglobin also differed significantly in favour of the non-nephropathy population according to the present data. This finding further supports the importance of good glycemic control to be one of the factors in delaying or preventing diabetes nephropathy.

Coming to the main two metabolic parameters of this present clinical study that is serum folic acid as well as vitamin B12; there was highly significant reduction in both levels among the patients belonging to the nephropathy group. The results closely matched with the earlier observation by Bherwan et al in two different studies conducted earlier ^[5, 7]. There are also some previous literatures indicating the association of folic acid deficiency with the complication of diabetes like DN ^[9, 10].

Medical literatures published earlier have shown the role of folic acid in important reactions like DNA methylation^[11] and conversion of homocysteine to methionine ^[12]. Therefore they have blamed hyperhomocysteinemia as a result of decreased folic acid levels to cause vascular damage to the kidneys and eyes leading to nephropathy among the diabetes mellitus patients. There are studies suggesting podocyte injury as a part of endothelial dysfunction resulting albuminuria in such patient population ^[13, 14].

Nitric oxide (NO) induced vasodilatation concept was also suggested by some previous authors [15, 16]. According to Schneider et al the active form of folic acid, 5 methyl tetrahydrofolate improves the nitric oxide synthetase (NOS) and thereby increases the concentration of vasodilators like nitric oxide (NO); which in turn reduces the endothelial dysfunction and retard nephropathy [17]. However, the same study failed to show any renal endothelial improvement in terms of reduction in albuminuria with high dose of folic acid supplementation.

In the case of Vitamin B12 the deficiency state can lead to accumulation of methylmalonic acid and homocysteine; both of which are toxic to the kidneys [18, 19]. But like folic acid, exogenous administration of Vitamin B12 neither showed any improvement in patients suffering from DN according to literature published earlier [20].

Another factor that could have played a role in Vitamin B12 deficiency is consumption of metformin among the patients with type2 diabetes mellitus, since the drug is known to produce the deficiency. However the estimation of actual extent of this effect was beyond the scope of the current analysis. The strengths of the present study lie in involvement of a comparatively larger sample size and applying the same methods of estimation of biochemical values. In recent times there was no such published report from the eastern part of India. Therefore it could provide a platform for the future researchers.

Conclusion

Based on the current clinical analysis Diabetic nephropathy patients have significantly lower levels of serum folic acid and vitamin B12 in comparison to those who did not develop nephropathy. They also displayed poor glycemic control besides overall poor renal status. But whether the exogenous administration of folic acid and or Vitamin B12 can improve the outcome of such patients would remain a topic of future research.

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