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ORIGINAL RESEARCH

To determine the serum Vitamin D, Vitamin B12, and Folic Acid levels in Diabetes without Nephropathy and in patients with Diabetes with Nephropathy.

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ABSTRACT

Background: Diabetes mellitus (DM) is a global health challenge characterized by chronic hyperglycemia resulting from impaired insulin secretion, action, or both. The interplay between metabolic and micronutrient imbalances in diabetes is complex, with significant roles played by vitamins and lipid metabolism in the pathophysiology of diabetic complications, including nephropathy. **Aim:** To determine the serum Vitamin D, Vitamin B12, and Folic Acid levels in Diabetes without Nephropathy and in patients with Diabetes with Nephropathy. **Materials & methods:** A cross-sectional pilot study was conducted at a tertiary care hospital, involving 200 diabetic patients divided into two groups: those with diabetes and nephropathy and those without nephropathy. The study required informed consent and excluded pregnant or lactating women, chronic illnesses, and vitamin supplement use. Serum samples were collected and tested for vitamin D and B12 levels. The study also estimated lipid profile parameters and used chemiluminescence immunoassays to measure vitamin D and B12 levels. The study aimed to understand the impact of diabetes on kidney function. **Results:** We observed significant differences (X^2 131.477; $P < 0.001$) when compared between the two groups with regards to urinary protein: creatinine ratio (≥ 1), Urine Pr Cr ratio (≥ 3), 24 h proteinuria ≥ 3.5 g/dL. Similarly, we observed significant differences when compared between the two groups with regards to serum protein (t=9.236, df=198, P <0.05), serum albumin (t=25.473; P < 0.05) and diastolic BP (t=26.636, df=198, P <0.05). We observed significant differences when compared between the two groups with regards to vitamin B12 (t=8.474, df=198, P <0.05), vitamin D (t=7.729, df=198, P <0.05), and HOMA-IR (t=23.696, df=198, P <0.05). **Conclusion:**The study highlights the crucial role of micronutrients like vitamin D, B12, and folic acid in diabetes development and complications, particularly nephropathy. Deficits in these vitamins contribute to oxidative stress, inflammation, and vascular dysfunction, and can be detected early by supplementation or dietary changes.

Key words: Diabetes mellitus, end stage renal disease, vitamin B12, Vitamin folic acid, vitamin D, protein, creatinine, urinary protein.

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INTRODUCTION

Diabetes mellitus (DM) is a global health challenge characterized by chronic hyperglycemia resulting from impaired insulin secretion, action, or both. Among its complications, diabetic nephropathy (DN) is a major cause of morbidity and mortality and is the leading contributor to end-stage renal disease (ESRD)[1-4]. The interplay between metabolic and micronutrient imbalances in diabetes is complex, with significant roles played by vitamins and lipid metabolism in the pathophysiology of diabetic complications, including nephropathy[5,6].

Vitamin D, a crucial regulator of calcium-phosphate homeostasis, has garnered attention for its immunomodulatory and anti-inflammatory properties, which are critical in preventing kidney damage[1]. Reduced serum Vitamin D levels are associated with insulin resistance, impaired glycemic control, and progression to diabetic nephropathy. Similarly, Vitamin B12 and folic acid, essential for methylation and homocysteine metabolism, influence vascular and renal health[2-4]. Deficiencies in these vitamins may exacerbate oxidative stress, endothelial dysfunction, and inflammation, contributing to the onset and progression of nephropathy[5,6].

This study aims to determine and compare the serum levels of Vitamin D, Vitamin B12, and folic acid in diabetic patients without nephropathy and those with

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diabetic nephropathy. Understanding the relationship between these parameters may provide valuable insights into their roles in the pathogenesis of nephropathy and aid in the identification of early markers and potential therapeutic targets for preventing disease progression [7-11].

MATERIALS & METHODS

After receiving ethics clearance from the Institutional Ethics Committee, a tertiary care hospital conducted a cross-sectional pilot study. We recruited patients from the outpatient and inpatient departments of endocrinology and nephrology. We enrolled 200 diabetic patients, dividing them equally into two groups: Group 1: 100 patients with diabetes and nephropathy (defined by albuminuria and/or reduced eGFR). Group 2: 100 patients with diabetes without nephropathy (normal renal function and absence of albuminuria). **Inclusion Criteria**: Adults aged 30–70 years. The individual must have been diagnosed with Type 2 Diabetes Mellitus for at least five years. The study requires the availability of informed consent. **Exclusion Criteria**: Pregnant or lactating women, patients with chronic illnesses other than nephropathy, and use of vitamin supplements within three months prior to the study.

5 ml of each person's fasting venous blood was drawn into flat containers in both groups using a disposable syringe and cannula in a clean room. After being separated from blood by centrifugation at 3000 rpm for 20 minutes, serum samples were aliquoted and stored at 20°C.We used a chemiluminescence immunoassay to measure the serum Vitamin D levels. A serum level of <20 ng/mL was considered vitamin D deficiency, 20–30 ng/mL is vitamin D insufficiency, and >30 ng/mL is vitamin D sufficiency.We used the enzyme-linked immunosorbent assay (ELISA) to determine the serum vitamin B12 levels. Standard cutoffs was used to categorize deficiency: <200 pg/mL is considered deficient, 200–300 pg/mL is equivocal, and >300 pg/mL is considered sufficient. Serum Folic Acid Measurement: We used a radioimmunoassay to measure folic acid levels, with levels below 3 ng/mL considered deficient. We estimated the following lipid profile parameters also.

Statistical analysis

The study used Microsoft Excel to analyze data, representing categorical variables as frequencies and percentages, and continuous variables as mean ± SD. The t test was used to compare diabetic patients with and without nephropathy, while the Chi-square test was used for categorical data. Statistical significance was with a p-value of less than 0.05.

RESULTS

Table 1: Laboratory Characteristics renal profile Details of the Study Population.

Parameter	Diabetes with nephropathy (n=100)	Diabetes without nephropathy (n=100)	
Urine Pr Cr ratio (≥ 1)	26	8	Degrees of freedom = 4
Urine Pr Cr ratio (≥ 3)	14	1	X^2 Test statistic = 131.477
24 h proteinuria ≥ 3.5 g/dL	10	1	P < 0.001
Renal Profile			**Student T test**
Serum Protein (g/dL)	4.12 ± 0.89	5.5 ± 1.2	P= 0.001 T= 9.236 Df = 198
Serum Albumin (g/dL)	2.1 ± 0.32	3.9 ± 0.63	P = 0.0001 T = 25.473 Df = 198
Serum Creatine (mg/dL)	4.9 ± 1.03	2.1 ± 0.21	P = 0.001 T = 26.636 Df = 198

In the present study (Table 1), renal profile details of the present study participants are given. We observed significant differences (X^2 131.477; P < 0.001) when compared between the two groups with regards to urinary protein: creatinine ratio (≥ 1), Urine Pr Cr ratio (≥ 3), 24 h proteinuria ≥ 3.5 g/dL. Similarly, we observed significant differences when compared between the two groups with regards to serum protein (t=9.236, df=198, P <0.05), serum albumin (t=25.473; P < 0.05) and diastolic BP (t=26.636, df=198, P <0.05).

Table 2: Laboratory details of vitamin B12, vitamin D, and folic acid in the study population.

Variable	Diabetes with nephropathy (n=100)	Diabetes without nephropathy (n=100)	P Value
Vitamin B12 (pg/ml)	167.1 ± 40.1	226.4 ± 55.9	= 0.0001 T = 8.474 Df = 198

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Vitamin D (pg/mL)	18.1 ± 9.1	26.4 ± 5.7	= 0.0001 T = 7.729 Df = 198
Folic acid (pg/ml)	4.5 ± 1.1	16.4 ± 4.9	= 0.0001 T = 23.696 Df = 198

In the present study (Table 2), laboratory details of the present study participants are given. We observed significant differences when compared between the two groups with regards to vitamin B12 (t=8.474, df=198, P <0.05), vitamin D (t=7.729, df=198, P <0.05), and HOMA-IR (t=23.696, df=198, P <0.05).

DISCUSSION

Diabetes mellitus significantly impacts kidney function, with notable differences in serum protein, serum creatinine, and serum albumin levels between patients with and without nephropathy[9,10]. Understanding these variations is crucial for early detection and management of diabetic kidney disease. In individuals with diabetes but without nephropathy, serum protein and albumin levels typically remain within normal ranges, reflecting intact kidney function[11]. However, the onset of diabetic nephropathy often leads to albuminuria, the presence of albumin in the urine, due to increased glomerular permeability[12]. This urinary loss can result in hypoalbuminemia (reduced serum albumin levels) and, in severe cases, progress to nephrotic syndrome, characterized by significant proteinuria (>3.5 g/day), hypoalbuminemia, hyperlipidemia, and edema[13]. Albuminuria is categorized based on the urinary albumin excretion rate: Microalbuminuria serves as an early marker of nephropathy, while macroalbuminuria indicates more advanced kidney damage[14].

Serum creatinine is a waste product filtered by the kidneys and serves as an indicator of renal function[15]. In diabetic patients without nephropathy, serum creatinine levels are usually within normal limits, corresponding to a normal estimated glomerular filtration rate (eGFR)[16]. As nephropathy progresses, declining kidney function leads to elevated serum creatinine levels and a reduced eGFR, signaling worsening renal impairment [17].

Regular monitoring of urinary albumin and serum creatinine is essential for early detection of diabetic nephropathy. The American Diabetes Association recommends annual screening for microalbuminuria in patients with type 1 diabetes of at least five years' duration and in all patients with type 2 diabetes starting at diagnosis[1-4]. Early identification allows for timely interventions, such as optimizing glycemic and blood pressure control, to slow disease progression and prevent complications. While serum protein, creatinine, and albumin levels remain stable in diabetic patients without nephropathy, the development of nephropathy leads to significant alterations in these parameters[5]. Regular assessment of these biomarkers is vital for the effective management of diabetes-related kidney disease[6].

Vitamin D plays a critical role in glucose metabolism by influencing insulin secretion and sensitivity. Several studies have demonstrated lower serum Vitamin D levels in diabetic patients, with significantly lower levels observed in those with nephropathy. The studies [16,17] highlighted that Vitamin D deficiency correlates with increased albuminuria and reduced estimated glomerular filtration rate (eGFR), emphasizing its role in diabetic kidney disease progression. Additionally, Vitamin D has anti-inflammatory and antifibrotic properties, which may mitigate the microvascular damage seen in nephropathy.

Vitamin B12 deficiency is prevalent in diabetic populations, particularly among those using metformin. This deficiency may exacerbate neuropathic complications in diabetes. In patients with nephropathy, the deficiency is further compounded by reduced renal clearance of homocysteine, leading to hyperhomocysteinemia. Studies, such as those by [18,19], suggest that B12 supplementation may help alleviate some of the metabolic complications associated with diabetes and nephropathy.

Folic acid, critical for homocysteine metabolism, is often reduced in diabetic patients. Lower folate levels contribute to hyperhomocysteinemia, a known risk factor for cardiovascular disease and nephropathy progression [20]. Research by [20,21] has demonstrated a significant association between reduced serum folate levels and increased risk of nephropathy in diabetic patients. This deficiency is particularly pronounced in those with advanced kidney disease due to impaired renal function and dietary restrictions.

CONCLUSION

This study emphasizes how important micronutrients, such as vitamin D, vitamin B12, and folic acid, are in the development of diabetes and its complications, especially nephropathy. Oxidative stress, inflammation, and vascular dysfunction, all of which contribute to renal impairment, significantly correlate with deficiencies in these vitamins. Comparing the levels in diabetic patients with and without nephropathy gives us a lot of information about how they work in the body and how they might be useful as early warning signs of how the disease is getting worse. Getting these deficiencies fixed by taking supplements or changing what you eat might be a good way to stop or slow down the development of diabetic nephropathy.

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Conflict of interest

There is no conflict of interest among the present study authors.

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