



## Some Biological Markers in Diabetic Patients

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### بعض المعلمات الحيوية في مرضى السكري

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## Abstract

Endstage renal disease (ESRD) usually comes on by diabetic nephropathy (DN), is one of the most dreaded chronic microvascular complications of diabetes. The objective of this study is to provide an overview of the DN-biomarker landscape with a particular emphasis on new approaches that improve the sensitivity of biomarkers for identifying patients who will develop DN or who are at risk of developing ESRD. This study was designed as a case-control study. 120 individuals were involved, divided into three groups a control group diabetic without nephropathy, Early stage and end stage of nephropathy, 40 patients was taken for each group identical in gender (20 male and 20 female).

All the diabetic patients included in the current study were diagnosed by nephrologist conducted in nephrology Clinic at Al-Emam Al Sadeq Hospital in Hila city and Department of Biochemistry in Collage of Medicine at University of Babylon from October 2022 to March 2023. C-reactive protein was determined by immune-detection method and Vit. D3 by chemico-luminescence immunoassay.

The results of current study revealed a significant increase in serum CRP, among diabetic nephropathy patients when compared to control and significant decrease of serum vitamin D3 in diabetic nephropathy patients than control.

**Keywords: Diabetic nephropathy (DN), Vitamin D, Diabetic complications and CRP.**



## المستخلص

يعد اعتلال الكلية السكري (DN) أحد أكثر مضاعفات مرضى السكري والسبب الرئيس لمرض الكلى في نهاية المرحلة (الفشل الكلوي بمراحله الأخيرة). و تلخص هذه الدراسة حالة العلامات الحيوية لـ DN مع التركيز على الاستراتيجيات الجديدة التي تعزز حساسية المؤشرات الحيوية للتنبؤ بحاله المرضى الذين سيطورون DN أو معرضون لخطر التقدم إلى الداء الكلوي بمراحله الأخيرة. صممت هذه الدراسة كدراسة حالة وضبط. شارك فيها 120 فرداً، مقسمين إلى ثلاث مجموعات. مجموعة ضابطة مصابة بمرض السكر بدون اعتلال الكلية، ومرحلة مبكرة ونهاية من اعتلال الكلية، حيث أخذ 40 مريضاً لكل مجموعة يكونوا متطابقين في الجنس (20 ذكراً و 20 أنثى). شخض جميع مرضى السكري المشمولين في الدراسة الحالية من قبل أخصائي أمراض الكلى في عيادة أمراض الكلى بمستشفى الإمام الصادق في مدينة الحلة وقسم الكيمياء الحيوية في كلية الطب بجامعة بابل من أكتوبر 2022 إلى مارس 2023. تم تحديد CRP بطريقة الكشف المناعي وقياس فيتامين D3 عن طريق المقايسة المناعية التلألؤ الكيمائي. كشفت نتائج الدراسة الحالية عن زيادة معنوية في CRP في مصل الدم لدى مرضى اعتلال الكلية السكري عند مقارنتها بمجموعة التحكم وانخفاض معنوي في فيتامين د 3 في مصل الدم لدى مرضى اعتلال الكلية السكري مقارنة بمرضى اعتلال الكلية السكري.

**الكلمات المفتاحية: اعتلال الكلية السكري، فيتامين د و التعقيبات**

**المصاحبة لمرض السكري.**



## Introduction

Diabetes mellitus (DM) is a metabolic disorder marked by inappropriate hyperglycemia that can be brought on by either an absolute or relative lack of insulin secretion from the beta cells of the Langerhans of the endocrine part of the pancreas, a decrease in the biologic effectiveness of insulin, or by problems with either or both the outer receptors. It is a growing concern and one of the most prevalent non-communicable diseases in the world. (Khalid, Petroianu, and Adem 2022). Polyuria, polydipsia, weight loss, occasionally polyphagia, and blurred vision are the typical signs of hyperglycemia (Adhi Raja, *et al.*, 2022). In most cases, the disease develops gradually, so the classic symptoms may go unnoticed by the patient in the early stages of the disease. All are indicators of elevated blood sugar. About 20–40% of diabetic patients experience a serious long-term complication, such as major microvascular complications of DM, diabetic kidney disease (DKD), or diabetic nephropathy (DN). (Samsu, 2021). Vitamin D is a fat-soluble vitamin. The significance of vitamin D in the prevention of diabetic nephropathy (DN) recently received special attention. Numerous scientific research and clinical trials had discovered a negative correlation of low level of vitamin D and DN risk (Rochel, 2022) and (Freedman and Towers, 1991). Blood plasma contains an annular (ring-shaped) protein called C-reactive protein (CRP). CRP is primarily produced in the liver as a result of factors released by macrophages and fat cells (adipocytes) after being stimulated by IL-6. It is an acute-phase protein. It is a member of the pentraxin protein family and interacts with lysophosphatidylcholine on the surface of bacteria and dead cells to activate the complement system through C1q and encourage phagocytosis. (Selen, Akoglu, and Agbaht, 2022), and (Ko, *et al.*, 2021).



## Materials and Methods

### Study design

This research was planned as a case-control study.

Fisher's exact test for sample size was used to determine sample size.

The patients were divided into three groups: 40 with diabetes who were receiving insulin treatment without complications, 40 with diabetes who were receiving insulin treatment along with a renal drug, and 40 with diabetes who were receiving insulin prescribed for dialysis prior to the first kidney wash. Subjects with T2DM, pregnancy, congestive heart failure, systemic lupus erythematosus, and polycystic kidney disease were excluded from the study.

### Population under investigation

The research featured 120 participants divided into three groups:

- 1) Group 1: 40 type 1 diabetes patients with early stage nephropathy (20 males and 20 females), the ages of the participants were 30 to 60 years.
- 2) Group 2: 40 type 1 diabetes patients with end-stage nephropathy (20 males and 20 females), the ages of the participants were 30 to 60 years.
- 3) group 3: 40 type 1 diabetes patients with normal renal function (20 males and 20 females) the ages of the participants were 30 to 60 years.

### Statistical analysis

To detect the effect of the various groups (end, early, and control) on research parameters, the Statistical Analysis System- SAS (2018) program was utilized. To make a meaningful comparison between means, the T-test



was performed. In this study, the correlation coefficient between variables was estimated, as well as the sensitivity and specificity of parameters in different groups (ROC analysis).

### **Ethical approval**

The study was carried out in accordance with the ethical standards set forth in the Helsinki Declaration. Before a sample was taken, it was done with the patient's verbal and analytical consent. According to document number 4, a local ethics committee reviewed and approved the study protocol, subject information, and consent form on 06/07/2022 to obtain this approval.

### **Techniques used to determine CRP and Vit. D3**

The concentration of CRP was measured using an a sandwich immune-detection method using AFFIAS-6 kit from Boditech. Med. Incorporated / Korea. The vitamin D3 concentration was determined by a competitive chemico-luminescence immunoassay using the Biomedical Engineering kit for the Maglumi 800 from Shenzhen New Industries.

## **Results**

### **Serum C- reactive protein among diabetic nephropathy patients and diabetic control group**

The current study's C- reactive protein results revealed highly significant differences ( $P \leq 0.01$ ) in early stage DN and end stage DN when compared to DM control group , Where the mean and SD are (  $4.34 \pm 0.17$ ,  $6.40 \pm 0.20$  ,  $2.27 \pm 0.12$  ) for the early , end stages diabetic nephropathy patients and DM



control respectively. Also a significant differences between the two patients groups ( early and end stage ) ( $P \leq 0.01$ ) were seen as illustrated in Table (1).

**Table (1): Serum C.R.P. mean and P values among studied groups.**

Parameters	Group	Mean $\pm$ SE	(P-value)
C.R.P. (mg/dl)	Early stage	4.34 $\pm$ 0.17	0.0001 **
	Control	2.27 $\pm$ 0.12	
	End stage	6.40 $\pm$ 0.20	0.0001 **
	Control	2.27 $\pm$ 0.12	
	Early stage	4.34 $\pm$ 0.17	0.0001 **
	End stage	6.40 $\pm$ 0.20	
** ( $P \leq 0.01$ )			

### Vitamin D3 among diabetes nephropathies patients group and control groups

According to the current study findings, diabetic nephropathy early and end stages patients group had significantly lower blood concentrations of vitamin D3 than the non-nephropathy diabetic control group ( $P$ -value  $\leq 0.001$ ), as shown in Table (2).

**Table (2): Mean of Vitamin D3 among diabetes nephropathies patients group and control groups**

Variable	Group	Mean $\pm$ SE	(P-value)
Vit. D3 (ng/ml)	Early stage	22.44 $\pm$ 1.06	0.0001 **
	Control	32.63 $\pm$ 0.78	
	End stage	11.58 $\pm$ 0.65	0.0001 **
	Control	32.63 $\pm$ 0.78	
	Early stage	22.44 $\pm$ 1.06	0.0001 **
	End stage	11.58 $\pm$ 0.65	
** ( $P \leq 0.01$ )			



The result of current study showed statistically significant negative correlation between vitamin D3 and CRP, R (-0.58). Figure (1).

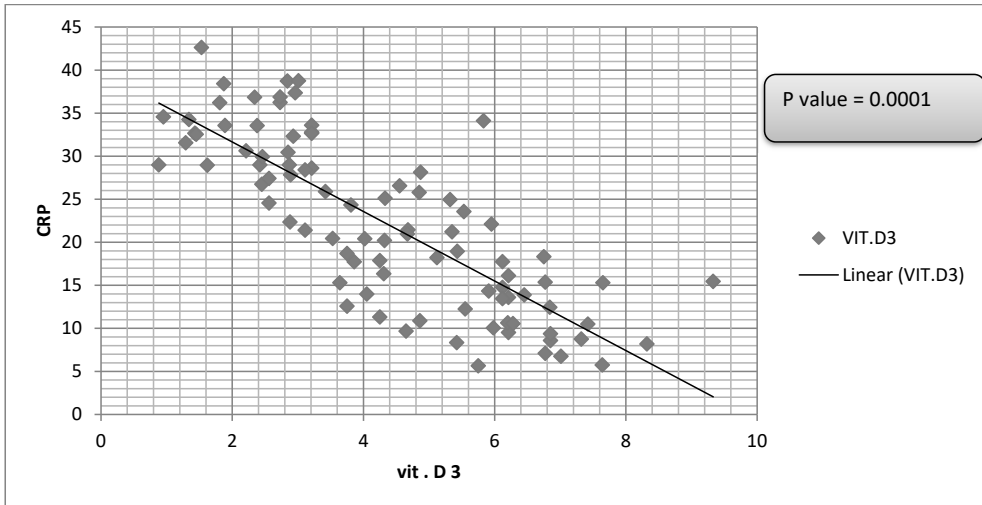


Figure (1): Correlation between Vitamin D3 and CRP in patients with diabetic nephropathy.

## Discussion

In response to inflammation, the class of proteins known as acute phase proteins experience changes in plasma concentration. An inflammatory basis for diabetes and its complications has garnered interest. Prior research had shown that inflammation was crucial in the pathogenesis of DM. C reactive protein (CRP), one of several markers of inflammation, is found to be important in diabetics. (Kandelouei, *et al.*, 2022). Abnormally high CRP levels have been linked to diabetic nephropathy because they can bind to a variety of biological substances and trigger complement. There has been interest in an inflammatory cause of diabetes and its complications, Hs-CRP is one of many markers of inflammation that is found to be significant in diabetics. As the "golden marker for inflammation," CRP, a pentameric





protein produced by the liver, may be linked to diabetic nephropathy. These findings are consistent with those of (. *et al.*, 2019), and (Zixin, *et al.*, 2022)

Many animal and human research suggest that vitamin D plays an important role in the development of DN. Nonetheless, the possibility of vitamin D's cyto-protective action and its influence on the reversal of pre-existing renal damage remains unproven. There are now a few ideas on the underlying biochemical and genetic pathways, including the association of vitamin D and inflammation, oxidative stress, and extracellular matrix aggregation. (Zixin, *et al.*, 2022). Table 2 shows that vitamin D3 levels in DN groups are lower than in control groups. Those findings are in agreement with (Hong, *et al.*, 2021) and (Naikawdi, Bharatha, and Hugar 2022). There was a statistically negative correlation between CRP and vit. D3 as illustrated in Figure (1). CRP increase in DNP because of chronic inflammation caused by hyper glycaemia this agree with (Sarhat and Khalaf, 2022), and (Rao, *et al.*, 2022). This study found a link between vitamin D deficiency and diabetic nephropathy. This result is consistent with earlier research suggesting that patients with chronic kidney disease have lower levels of 1 $\alpha$ ,25-(OH)2D3 and may develop frank rickets or osteomalacia as a result of a 1 $\alpha$ ,25-(OH)2D3 deficiency brought on by a lack of renal 1 $\alpha$ -hydroxylase. (Mohandes, *et al.*, 2023).

## Conclusion

It is it possible to conclude that VIT. D3 is correlated with progression of DNP and it might be used as one of the most reliable biomarkers for predicting renal function. CRP is an inflammatory marker and can be used to predict progression of diabetic nephropathy.



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