

## Decreased Serum 25-Hydroxycalciferol Levels in Pre-diabetic Adults

Qurrat-ul-Ain<sup>1</sup>, Dilshad Ahmed Khan<sup>1</sup>, Aamir Ijaz<sup>1</sup>, Farooq Ahmad Khan<sup>2</sup> and Atif Latif<sup>3</sup>

### ABSTRACT

**Objective:** To determine the serum 25-hydroxycalciferol levels [25(OH)D] in adults with pre-diabetes and normoglycaemia to examine a possible association of vitamin D deficiency with pre-diabetes.

**Study Design:** Case control study.

**Place and Duration of Study:** Armed Forces Institute of Pathology, Rawalpindi, from November 2012 to July 2013.

**Methodology:** A total of 272 adults including 136 pre-diabetics and 136 normoglycaemics of either gender aged 20 years and above were consecutively inducted. Patients with diabetes mellitus, pregnancy, rickets and osteomalacia, ischemic heart disease, chronic kidney disease and chronic liver disease were excluded. Fasting Plasma Glucose (FPG) was estimated with hexokinase method on Modular p800 Roche chemistry analyzer while serum 25(OH)D was measured on Diasorin Liaison immunoassay analyzer using the chemiluminescent technique. Mean 25(OH)D levels in pre-diabetic and normoglycaemic groups were compared using Mann-Whitney U test. Spearman's correlation coefficient ' $r_s$ ' was determined between serum 25(OH)D and FPG. Odds ratio for vitamin D deficiency was also calculated.

**Results:** Mean serum 25(OH)D level was low in pre-diabetics (23.2 nmol/L) as compared to normoglycaemics (29 nmol/L;  $p=0.001$ ). Serum 25(OH)D level had inverse correlation with FPG ( $r_s = -0.448$ ,  $p=0.000$ ). There was also significant association of vitamin D deficiency with pre-diabetes compared with normoglycaemia (OR: 2.21,  $p= 0.016$ ; 95% CI: 1.15-4.27).

**Conclusion:** Vitamin D deficiency with pre-diabetes suggested that vitamin D may have an important role in pathogenesis of pre-diabetes.

**Key Words:** Pre-diabetes. Vitamin D deficiency. Fasting plasma glucose. Chemiluminescent technique.

### INTRODUCTION

Pre-diabetes is the stage of Impaired Fasting Glucose (IFG) and/or Impaired Glucose Tolerance (IGT) seen much earlier in natural history of diabetes mellitus type 2 (T2DM).<sup>1</sup> The disease has substantial implications on economy and health due to 3 - 10 fold increased risk of T2DM and development of serious morbidities like coronary heart disease, retinopathy and neuropathy.<sup>2,3</sup> An alarming rise in the magnitude of the disease in recent years is probably due to sedentary lifestyle, obesity and lack of exercise. Pre-diabetes commonly associates with metabolic syndrome. Both share a common metabolic soil having insulin resistance and systemic inflammation engendered by obesity as major contributors.<sup>4</sup> Though vitamin D is considered essential for maintenance of bone health, its role has also been implicated in glucose metabolism, mainly through facilitation of insulin secretion due to presence of

Vitamin D Receptor (VDR) on pancreatic  $\beta$  cells, immune modulation and increased expression of insulin receptors.<sup>5,6</sup> Most studies have reported an inverse association between vitamin D status and hyperglycaemia in various populations.<sup>7-9</sup> Vitamin D deficiency is pandemic worldwide with a prevalence of 70 - 90% in Pakistan.<sup>10</sup> Major causes of vitamin D deficiency are unusually low sun exposure, lack of vitamin D fortified foods and malabsorption.

Despite high prevalence of both pre-diabetes and vitamin D deficiency in Pakistan, data relating to the two disorders is very scarce. This may have important public health implications like inclusion of pre-diabetes in high risk groups for vitamin D deficiency who require vitamin D estimation and also provide strong evidence for future research to establish their casual relationship.

The study was aimed to compare mean serum 25(OH)D levels in adults with pre-diabetes and normoglycaemia to find any association between these two highly prevalent disorders in our population.

### METHODOLOGY

This case control study was conducted at the Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology, Rawalpindi from November 2012 to July 2013 after approval from the Ethical Committee of the Institute. One hundred and thirty-six individuals were consecutively selected in each

<sup>1</sup> Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology (AFIP), Rawalpindi.

<sup>2</sup> Department of Chemical Pathology, Rai Medical College, Sargodha.

<sup>3</sup> Department of Radiology, PNS Hafeez, Islamabad.

Correspondence: Dr. Qurrat-ul-Ain, Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology, Rawalpindi.

E-mail: qurrat\_78@hotmail.com

Received: January 27, 2015; Accepted: September 10, 2015.

group. Adults of either gender, aged more than 20 years, were included in the study. All the participants gave written informed consent. Subjects with FPG level 5.6 - 6.9 mmol/L were recruited in case (pre-diabetes) group while those with FPG level < 5.6 mmol/L were enrolled in control (normoglycaemia) group. Individuals with known diabetes mellitus, pregnancy, rickets and osteomalacia, IHD, chronic kidney disease and chronic liver disease were excluded from the study.

Three ml of blood samples were taken after an overnight fast by venipuncture in sample tube with NaF/EDTA for FPG and in gel tube for serum 25(OH)D. FPG was analyzed within 2 hours of sample collection. Samples for 25(OH)D were allowed to clot, serum was separated by centrifuging at 3000xg and stored at -80°C until biochemical analysis. FPG was analyzed by Roche reagent kit based on hexokinase method on Modular p800 Roche chemistry analyzer. Serum 25(OH)D was analyzed on Diasorin Liaison immunoassay analyzer using competitive chemiluminescent technique.

All statistical analyses were done using Statistical Package for Social Sciences version 20 (SPSS Inc., Chicago, IL, USA). Descriptive statistics for qualitative variables like gender were determined as percentages. Mean and SD or median with interquartile range were calculated for quantitative variables like age, weight, height, BMI, FPG and vitamin D. Mean vitamin D level was compared between pre-diabetes and normoglycaemia groups using Mann-Whitney U-test. Vitamin D deficiency was defined as serum 25(OH)D level below 50 nmol/L. Chi-square test and odds ratio were also calculated to evaluate relationship

of vitamin D deficiency with pre-diabetes. At 95% confidence interval, p-value less than 0.05 was considered as significant.

## RESULTS

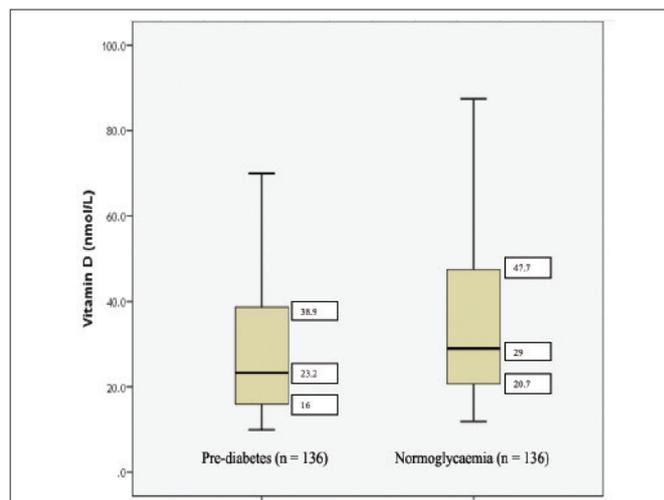
One hundred and thirty-six subjects and controls were consecutively selected from the individuals reporting for FPG levels at the reception of AFIP, Rawalpindi. They were recruited in pre-diabetes and normoglycaemia groups on the basis of FPG levels after careful scrutiny for exclusion criteria.

As shown in Table I, 70 (51%) among pre-diabetics were males and 66 (49%) were females. Though there were no significant difference in age, weight and mean BMI of both groups, subjects with pre-diabetes had a significantly lower mean 25(OH)D level as compared to normoglycaemics (p=0.001; Figure 1). Serum 25(OH)D levels in pre-diabetics with vitamin D deficiency (serum 25(OH)D<50 nmol/L) were also significantly low as compared to controls (p=0.006; Figure 2).

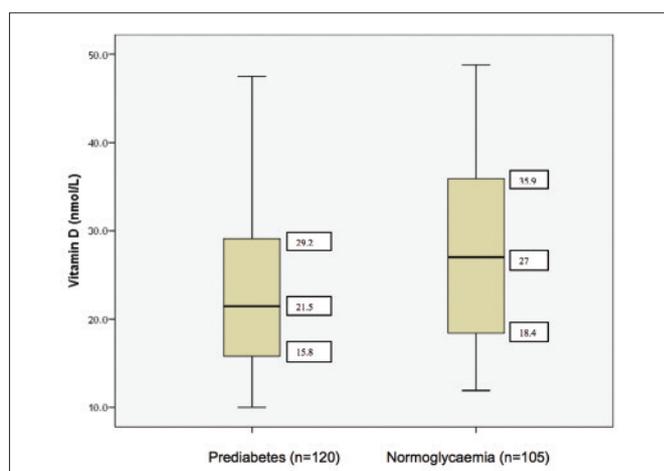
**Table I:** Baseline characteristics in healthy and pre-diabetes adults (N=272).

Parameter	Normoglycaemia	Pre-diabetes	p-value
Number (n)	136	136	
Male, n (%)	75 (55)	70 (51)	
Age (years)			
Median (Interquartile range)	49 (40 - 59)	52(43 - 60)	0.08
Smoking, n (%)			
No	83 (61)	78 (57)	
Yes	53 (39)	58 (43)	
Weight (kg)			
Median (Interquartile range)	70 (64 - 78)	72 (66 - 78)	0.098
BMI (kg/m <sup>2</sup> )			
Median (Interquartile range)	25 (23 - 26)	25 (24 - 26)	0.307
FPG, mmol/L			
Median (Interquartile range)	4.7 (4.1-5.1)	6.1 (5.8-6.3)	0.000
Serum vit D, nmol/L			
Minimum	11.9	10	
Maximum	87.5	70	
Median (Interquartile range)	29 (29.7 - 47.7)	23.2 (16 - 38.9)	0.001
Serum vit D in adults with vit D deficiency, nmol/L			
Minimum	11.9	10	
Maximum	48.8	47.5	
Median (Interquartile range)	27 (18.35 - 35.9)	21.5 (15.8 - 29.2)	0.006

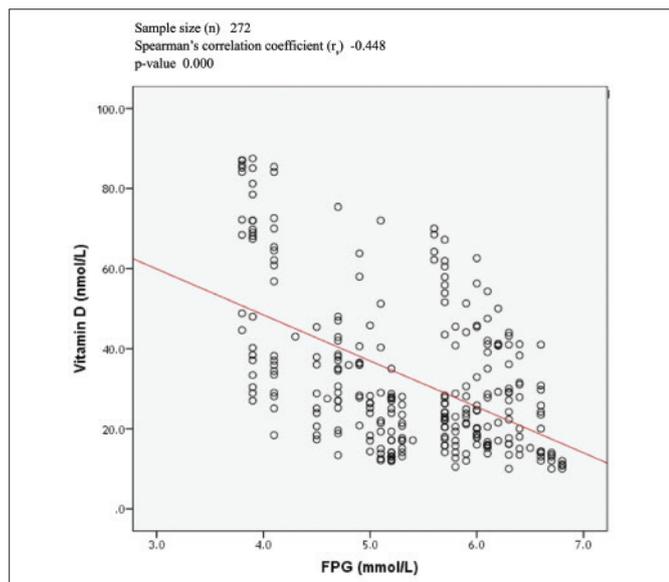
vit D = vitamin D; FPG = fasting plasma glucose; BMI = body mass index



**Figure 1:** Box and Whisker plot showing median (interquartile range) for serum vitamin D levels in adults with pre-diabetes and normoglycaemia.



**Figure 2:** Box and Whisker plot showing median (interquartile range) for serum vitamin D levels in vitamin D deficient adults with pre-diabetes and normoglycaemia.



**Figure 3:** Scatter plot showing the inverse relationship between fasting plasma glucose (mmol/L) and serum vitamin D (nmol/L).

A significant inverse correlation was also seen between serum vitamin D and FPG levels ( $r_s = -0.448$ ,  $p = 0.000$ ) as shown in Figure 3. The odd ratio for vitamin D deficiency in normoglycaemia and pre-diabetes groups was 2.21 ( $p = 0.016$ , 95% CI: 1.15 - 4.27) establishing a possible association of vitamin D deficiency with pre-diabetes.

## DISCUSSION

Pre-diabetes is not a distinctive clinical entity but a stage in the natural history of disorder of glucose metabolism which is an important risk factor not only for T2DM but also for cardiovascular and microvascular complications related to T2DM, like nephropathy and retinopathy.<sup>11</sup> Both components of pre-diabetes, IFG and IGT, are frequently related with other cardiovascular risk factors like abdominal or visceral obesity, dyslipidemia particularly high triglyceride levels and/or low HDL cholesterol levels and hypertension; a disorder called metabolic syndrome.<sup>12,13</sup> Thus halting the disease progression at pre-diabetes stage will not only delay development of T2DM but also decrease morbidity and mortality due to micro- and macro-vascular complications. Though the best approach in this regard is lifestyle modification, but it is difficult to achieve and maintain for long periods.<sup>14,15</sup> The contribution of inadequate vitamin D levels to various aspects in the pathogenesis of T2DM, e.g. pancreatic beta-cell dysfunction, impaired insulin secretion and insulin resistance, has brought interest among researchers to find its association with pre-diabetes.<sup>16</sup>

The association between vitamin D deficiency and pre-diabetes has not yet been evaluated in Pakistan. This study provides estimates of vitamin D status in a representative sample of our adult population and

reports association between 25(OH)D levels and pre-diabetes. It has revealed the following significant findings: First, lower serum 25(OH)D levels were positively associated with pre-diabetes in adults. Second, there is more risk of developing pre-diabetes in adults with vitamin D deficiency. Third, serum 25(OH)D has inverse correlation with FPG.

The present findings are consistent with the findings of Scragg *et al.* who revealed low vitamin D levels in adults with IGT compared with normoglycaemia ( $p = 0.0016$ ).<sup>17</sup> Similar results were also found in a study on American Arabs by Pinelli, which revealed that 25(OH)D levels were lower in men with glucose intolerance than in those who were normoglycaemic ( $p = 0.005$ ).<sup>18</sup> However, data from NHANES III revealed ethnic differences as shown by an inverse association between 25(OH)D and undiagnosed diabetes among non-Hispanic whites [OR:1.00,0.25(0.11-0.6)] and Mexican Americans [OR:1.00,0.17(0.08-0.37)]; but no association among non-Hispanic blacks [OR:1.00,3.4(1.07-10.86)]. Analysis of data from NHANES III also showed that only non-Hispanic whites have negative relation between 25(OH)D and pre-diabetes ( $p = 0.0001$ ). In addition to ethnic differences noted in NHANES III, a paradox relation between vitamin D and glycaemia was also reported in females.<sup>19</sup> A large Finnish cohort of 7503 adults has revealed that the higher baseline 25(OH)D level reduced the risk of incident diabetes by 72% in men but not in women after adjustment for type 2 DM risks.<sup>20</sup> A study by Baynes *et al.* on elderly population has also revealed no association between 25(OH)D and PFG as a component of metabolic syndrome.<sup>17</sup> The discordant outcomes of these observational studies can be explained by the heterogeneity of the participants, e.g. differences in race, BMI, seasonal variation and blood glucose levels. Interaction of various confounders can also play a role; for instance, obesity may predispose subjects to both vitamin D insufficiency and pre-diabetes.<sup>17,21</sup>

We have also demonstrated that comparison of mean serum 25(OH)D after using cutoff of 50 nmol/L to diagnose vitamin D deficiency also revealed significantly lower levels in pre-diabetics with vitamin D deficiency as compared to controls ( $p = 0.006$ ). Similar results were also reported by Shanker *et al.* who, after categorization of vitamin D into 4 quartiles, revealed that lower serum 25(OH)D levels were positively associated with pre-diabetes and this association was independent of confounding factors like age, gender, ethnicity, BMI, seasonal variation, hypertension etc.<sup>22</sup>

The finding of lower mean 25(OH)D levels in pre-diabetes and a positive association of vitamin D deficiency with pre-diabetes highlights the physiological role of vitamin D in glucose homeostasis. However, the effects of vitamin D deficiency on glucose metabolism appear to depend on many factors known to affect

vitamin D and glucose metabolism like ethnic and/or genetic background, gender, obesity, and comorbidities.<sup>21</sup>

More recently a very strong evidence regarding association of serum 25(OH)D levels and pre-diabetes has been provided by the prospective cohort study on participants of the Copenhagen City Heart Study and meta-analysis by Afzal, Bojesen and Nordestgaard. They observed increasing risk of T2DM with decreasing 25(OH)D, even after correction of confounders like BMI, smoking, seasonal variation, physical activity and socio-economic status. Their meta-analysis has also revealed that low 25(OH)D levels are strongly associated with increased risk of T2DM irrespective of population, level of adjustment, or study design.<sup>23</sup> This is by far the largest meta-analysis on this subject as it included all prospective, cohort and nested case control studies published until July 2012.<sup>23</sup> This meta-analysis further supports the findings of this study.

There are certain limitations in this study like lack of adjustments for some important confounders (sunlight exposure, physical activity), random error due to use of single vitamin D measurement, and observational study design, which make it nearly impossible to ascertain the causal relationship between vitamin D status and pre-diabetes.

## CONCLUSION

The serum mean vitamin D levels were low in adults with pre-diabetes compared with normoglycaemia. An increasing risk of pre-diabetes was also observed in adults with vitamin D deficiency. Pre-diabetes can be included in high risk groups for vitamin D deficiency who require vitamin D estimation. However, prospective and randomized control trials are needed before supplementation with vitamin D can be recommended to help in prevention or slowing the progression of pre-diabetes to diabetes.

## REFERENCES

1. American Diabetes Association. Standards of medical care in diabetes. *Diabetes Care* 2013; **36** (Suppl 1):S11-66.
2. Garber AJ, Handelsman Y, Einhorn D, Bergman DA, Bloomgarden ZT, Fonseca V, *et al.* Diagnosis and management of prediabetes in the continuum of hyperglycemia: when do the risks of diabetes begin? A consensus statement from the American College of Endocrinology and the American Association of Clinical Endocrinologists. *Endocr Pract* 2008; **14**:933-46.
3. Zhang Y, Dall TM, Chen Y, Baldwin A, Yang W, Mann S, *et al.* Medical cost associated with prediabetes. *Popul Health Manag* 2009; **12**:157-63.
4. IDF. Diabetes Atlas. 6th edition. 2013; 29-32.
5. Sung CC, Liao MT, Lu KC, Wu CC. Role of vitamin D in insulin resistance. *J Biomed Biotechnol* 2012; **2012**:634195.
6. Alvarez JA, Ashraf A. Role of vitamin D in insulin secretion and

- insulin sensitivity for glucose homeostasis. *Int J Endocrinol* 2010; **2010**:351385.
7. Chiu KC, Chu A, Go VL, Saad MF. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. *Am J Clin Nutr* 2004; **79**:820-5.
8. Forouhi NG, Luan J, Cooper A, Boucher BJ, Wareham NJ. Baseline serum 25-hydroxy vitamin D is predictive of future glycemic status and insulin resistance: The Medical Research Council Ely Prospective Study 1990-2000. *Diabetes* 2008; **57**: 2619-25.
9. Liu S, Song Y, Ford ES, Manson JE, Buring JE, Ridker PM. Dietary calcium, vitamin D, and the prevalence of metabolic syndrome in middle-aged and older U.S. women. *Diabetes Care* 2005; **28**:2926-32.
10. Iqbal R, Khan AH. Possible causes of vitamin D deficiency (VDD) in Pakistani population residing in Pakistan. *J Pak Med Assoc* 2010; **60**:1-2.
11. Diabetes Prevention Program Research Group. Impact of intensive lifestyle and metformin therapy on cardiovascular disease risk factors in the Diabetes Prevention Program. *Diabetes Care* 2005; **28**:888-94.
12. Mitri J, Muraru MD, Pittas AG. Vitamin D and diabetes mellitus type 2: a systematic review. *Eur J Clin Nutr* 2011; **65**:1005-15.
13. Nathan DM, Davidson MB, DeFronzo RA, Heine RJ, Henry RR, Pratley R, *et al.* Impaired fasting glucose and impaired glucose tolerance. *Diabetes Care* 2007; **30**:753-9.
14. Chimaroke E, Dagogo-Jack S. Understanding and identifying prediabetes: can we halt the disease? *US Endocrinology* 2008; **4**:20-3.
15. Pour OR, Dagogo-Jack S. Prediabetes as a therapeutic target. *Clin Chem* 2011; **57**:215-20.
16. Borissova AM, Tankova T, Kirilov G, Dakovska L, Kovacheva R. The effect of vitamin D3 on insulin secretion and peripheral insulin sensitivity in type 2 diabetic patients. *Int J Clin Pract* 2003; **57**:258-61.
17. Barengolts E. Vitamin D role and use in prediabetes. *Endocr Pract* 2010; **16**:476-85.
18. Pinelli NR, Jaber LA, Brown MB, Herman WH. Serum 25-hydroxy vitamin D and insulin resistance, metabolic syndrome, and glucose intolerance among Arab Americans. *Diabetes Care* 2010; **33**:1373-5.
19. Scragg R, Sowers M, Bell C. Serum 25-hydroxy-vitamin D, diabetes, and ethnicity in the Third National Health and Nutrition Examination Survey. *Diabetes Care* 2004; **27**:2813-8.
20. Knekt P, Laaksonen M, Mattila C, Härkänen T, Marniemi J, Heliövaara M, *et al.* Serum vitamin D and subsequent occurrence of diabetes mellitus type 2. *Epidemiology* 2008; **19**: 666-71.
21. Pittas AG, Nelson J, Mitri J, Hillman W, Garganta C, Nathan DM, *et al.* Plasma 25-hydroxy-vitamin D and progression to diabetes in patients at risk for diabetes: an ancillary analysis in the Diabetes Prevention Program. *Diabetes Care* 2012; **35**: 565-73.
22. Shankar A, Sabanayagam C, Kalidindi S. Serum 25-hydroxy-vitamin D levels and prediabetes among subjects free of diabetes. *Diabetes Care* 2011; **34**:1114-9.
23. Afzal S, Bojesen SE, Nordestgaard BG. Low 25-Hydroxy-vitamin D and risk of diabetes mellitus type 2: a prospective cohort study and meta-analysis. *Clin Chem* 2013; **59**:381-91.

