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Inflammation and its Association with Vitamin D in Newly Diagnosed Type 2 Diabetes 1

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I. Introduction

The prevalence of diabetes is rapidly rising all over the globe at an alarming rate. [1]

Over the past 30 years, the status of diabetes has changed from being considered as a mild disorder of the elderly to one of the major causes of morbidity and mortality affecting even the youth and middle-aged people. It is important to note that the rise in prevalence is seen in all six inhabited continents of the globe. Although there is an increase in the prevalence of type 1 diabetes also, the major driver of the epidemic is the more common form of diabetes, namely type 2 diabetes, which accounts for more than 90 per cent of all diabetes cases. Nowhere is the diabetes epidemic more pronounced than in India as the World Health Organization (WHO) reports show that 32 million people had diabetes in the year 2000. [2]

The International Diabetes Federation (IDF) estimates the total number of diabetic subjects to be around 40.9 million in India and this is further set to rise to 69.9 million by the year 2025. [3]

Inflammation is a protective mechanism of the body. However, in chronic diseases like, diabetes mellitus, hypertension, asthma etc., this protective mechanism becomes an important mechanism for progression of disease. In Type 2 diabetes mellitus the decreasing β cell mass is also associated with glucose toxicity mediated through IL-1β induced apoptosis. There are many studies emphasizing the presence and importance of the inflammatory component in the pathogenesis of Diabetes mellitus. A very important role is played by adipose tissue, which releases various pro-inflammatory cytokines, such as, tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6), C Reactive Protein (hs CRP), intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM1). [4,5,6]

Vitamin D deficiency has been shown to alter insulin synthesis and secretion in both humans and animal models. It has been reported that vitamin D deficiency may predispose to glucose intolerance, altered insulin secretion and type 2 diabetes mellitus. Vitamin D replenishment improves glycemia and insulin secretion in patients with type 2 diabetes with established hypovitaminosis D, thereby suggesting a role for vitamin D in the pathogenesis of type 2 diabetes mellitus. The presence of vitamin D receptors (VDR) and vitamin D–binding proteins (DBP) in pancreatic tissue and the relationship between certain allelic variations in the VDR and DBP genes with glucose tolerance and insulin secretion have further supported this hypothesis. The mechanism of action of vitamin D in type 2 diabetes is thought to be mediated not only through regulation of plasma calcium levels, which regulate insulin synthesis and secretion, but also through a direct action on pancreatic b-cell function. [7]

Deficiency of vitamin D has been associated with increased risk of developing Type 2 diabetes mellitus (DM) and cardiovascular diseases. [8]

Vitamin D deficiency is highly prevalent in India. About 70% of adults in both rural and urban areas were found showing manifestations of vitamin D deficiency. [9,10]

However, literature search shows that the data regarding the status of vitamin D in adult Indian population is scarcely available. Since, both Type 2 diabetes and deficiency of vitamin D are highly prevalent in Indian population, therefore, the study was designed to assess the vitamin D status of the study population by measuring serum 25(OH) D levels, and its association with inflammatory markers in newly diagnosed type 2 diabetes mellitus.

II. Material And Methods

This cross-sectional study was carried out with two groups. Group 1 (n=147): Newly diagnosed type 2 diabetics or the Cases and Group 2 (n=147): Apparently healthy individuals or the Controls.

Newly diagnosed type 2 diabetics of both sexes, in the age group between 18-35 years with Fasting blood glucose ≥ 126mg/dl and with symptoms of diabetes mellitus-polyuria, polydipsia, fatigue, weight loss were include as cases.

Known diabetics and those with metabolic syndrome, morbid obesity, thyroid dysfunctions and any other condition which altered the glucose homeostasis were excluded from our study.

Prior to the commencement of our study, clearance from institutional ethical committee was obtained followed by written and informed consent from all the participants in the study.

Estimation of Vitamin D and blood glucose levels was done in Central Clinical Laboratory of the Department of Biochemistry, Jorhat Medical College and Hospital, Jorhat whereas the cytokine levels of the same study population was carried out in the NABL accredited laboratory of Ayursundra Hospital, Jorhat .

The patients were asked to refrain from heavy physical activity for 24 hours and to abstain from consumption of alcohol and caffeinated beverages for 12 hours prior to the measurements.

The temperature of the laboratory was kept between 25^{o} C - 28^{o} C and lights subdued.

The patients were asked to void urine before testing and made to sit in the lab comfortably to accustom to the new environment.

Baseline and anthropometric parameters were recorded before blood collection.

Blood was collected by venipuncture. 5ml of blood was collected and allowed to clot. Serum was separated and stored in refrigerator to estimate the inflammatory markers and vitamin D levels separately.

Height was measured by using a stadiometer in the upright position and weight measured on a weighing machine. BMI was calculated by weight (Kg) divided by the square of height in meters.

After a 30-minute acclimatization period, BP was measured 3 times to the nearest of 2 mm Hg in the sitting position, using a mercurial sphygmomanometer and appropriately sized cuffs. The average of 3 measurements was used to calculate systolic and diastolic BPs; mean BP was calculated as the diastolic value plus one third of the pulse pressure value.

Estimation of vitamin D levels and inflammatory markers were carried out by commercially available kits.

Statistical Analysis

Statistical analysis was carried out by using R for windows. The data was expressed as Mean ± SD. Normality was tested with Kolmogorov – Smirnov test. To study the between group differences, independent t test was used. To study the association of vitamin D levels with inflammatory markers Pearson's correlation was used. The null hypothesis will be rejected at $P \leq 0.05$.

III. Results

The baseline and anthropometric parameters of cases and controls were given in Table 1.

Table-1: Baseline characteristics of controls and newly diagnosed type 2 diabetes.

Sl. No	Parameter	Controls(n=147) Mean ±SD	Cases (n=147) Mean ±SD	P -value
1	Age in years	46.04 ± 4.30	46.37 ± 4.30	0.5160
2	Height in cm	171.80 ± 6.20	171.85 ± 6.31	0.9410
3	Weight in kg	68.19 ± 5.67	76.87 ± 5.44	0.0001
4	BMI kg/m^2	23.16 ± 2.32	26.05 ± 1.67	0.0001
5	Gender	108/39	112/35	0.0001
6	SBP (mm of Hg)	113.76 ± 6.00	125.04 ± 4.55	0.0001
7	DBP (mm of Hg)	79.46 ± 3.00	80.85 ± 2.98	0.0001
8	PP (mm of Hg)	34.30 ± 6.00	44.19 ± 3.92	0.0001
9	MAP (mm of Hg)	90.89 ± 3.15	95.58 ± 3.06	0.0001
10	RPP	8880.18 ± 563.92	9910.09 ± 455.79	0.0001
11	HR (bpm)	78.05 ± 2.75	79.25 ± 2.49	0.0001

BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic pressure, PP: Pulse pressure, MAP: Mean arterial pressure, RPP: Rate pressure product, HR, Heart rate.

As shown in Table 1, there were no significant differences in the age ($p< 0.516$) and height ($p < 0.941$) of the study participants.

However, significant differences in weight ($p < 0.001$), BMI ($p < 0.001$), Heart rate ($p < 0.001$), blood pressure (SBP $p < 0.001$, DBP $p < 0.001$) and rate pressure product ($p < 0.001$) were observed.

Table 2 shows the comparison of Vitamin D and FBG in cases and controls

Table-2: Vitamin D and fasting blood glucose levels in cases and controls

Sl. No	Parameter	Controls(n=147) Mean ±SD	Cases (n=147) Mean ±SD	P -value
1	Vitamin D (ng/ml)	19.19 ± 2.08	10.47 ± 2.13	<0.0001
2	FBG (mg/dl)	95.42 ± 6.06	132.70 ± 3.88	<0.0001

FBG: Fasting blood glucose.

Vitamin D levels were significantly ($p < 0.001$) lower, whereas the FBG levels were significantly ($p < 0.001$) higher in cases when compared to controls.

The inter group comparison of TNF alpha, IL 1 beta, IL 6, ICAM 1, VCAM 1 and hsCRP are depicted in Table 3.

Table-3: Inflammatory cytokine levels of cases and controls

Sl. No	Parameter	Controls(n=147) Mean ±SD	Cases (n=147) Mean ±SD	P -value
1	TNF alpha (pg/ml)	143.58 ± 52.75	289.93 ± 111.74	<0.0001
2	IL 1 beta (pg/ml)	6.13 ± 1.76	15.80 ± 1.73	<0.0001
3	IL 6 (pg/ml)	4.86 ± 0.70	13.23 ± 1.27	<0.0001
4	ICAM 1 (ng/ml)	10.42 ± 1.74	29.94 ± 4.36	<0.0001
5	VCAM 1 (ng/ml)	10.25 ± 1.57	29.47 ± 4.02	<0.0001
6	hsCRP (ng/ml)	2716.38 ± 838.77	9106.24 ± 2362.02	<0.0001

TNF alpha: Tumor necrosis factor alpha, IL 1 beta: Interleukin 1 beta, IL 6: Interleukin 6, ICAM 1: Intracellular cell adhesion molecule 1, VCAM 1: Vascular cell adhesion molecule 1, hsCRP: high sensitivity C reactive protein.

The levels of TNF alpha ($p < 0.001$), IL 1 beta ($p < 0.001$), IL 6 ($p < 0.001$), ICAM 1 ($p < 0.001$), VCAM 1 ($p < 0.001$) and hsCRP ($p <0.001$) were significantly higher in cases when compared to controls.
The levels of Vitamin D had a significantly negative correlation with the inflammatory markers TNF alpha ($r = -0.79$; $p < 0.001$), IL 1beta ($r = -0.78$; $p < 0.001$), IL 6 ($r = -0.70$; $p < 0.001$), hs CRP ($r = -0.89$; $p < 0.001$), ICAM 1 ($r = -0.82$; $p < 0.001$), VCAM 1 ($r = -0.81$; $p < 0.001$) in cases.

IV. Discussion

Significantly difference in weight ($p < 0.000$), BMI ($p < 0.000$), Heart rate ($p < 0.000$), blood pressure (SBP $p < 0.000$, DBP $p < 0.000$) and rate pressure product ($p < 0.000$) and pulse pressure ($p < 0.000$) were observed. In developed countries, T2DM mostly affects elderly; but in developing countries like India, the working lives of the younger population are affected causing threat to their health. [11]

Diabetic patients often feel challenged by their disease, day-to-day management and its substantial demands. Diabetics have higher risk for cardiovascular disease (CVD) and metabolic dysfunctions. Although the management of diabetes and its impacts is expensive, cost effective measures prevent their occurrence. Instead of merely aiming at the glycemic control, paying attention to the reduction of cardiovascular risk in diabetes is the most compelling aspect of management for the risk reduction globally. [12]

Very few studies have addressed the relationship between weight change, closely associated with BMI, and incidence of CVD in diabetes patients. In our study, the weight and BMI were significantly high in newly diagnosed type 2 diabetics. The blood pressure and heart rate were also significantly high in newly diagnosed type 2 diabetics. Further, the rate pressure product (which is noninvasive myocardial oxygen consumption marker) is increased which indicates the cardiovascular risk in newly diagnosed type 2 diabetics. CVD is elevated in type 2 diabetes mellitus due to a complex combination of various traditional and non-traditional risk factors, that have an important role to play in the beginning and the evolution of atherosclerosis over its long natural history from endothelial function to clinical events. [13].

Vitamin D levels were significantly low ($p < 0.001$), whereas the FBG levels were significantly high in newly diagnosed type 2 diabetics when compared to controls ($p < 0.001$).

The role of vitamin D in calcium and phosphorous homeostasis and bone metabolism is well understood. However, more recently, vitamin D and calcium homeostasis have also been linked to a number of conditions, such as neuromuscular function, cancer, and a wide range of chronic diseases, including autoimmune diseases, atherosclerosis, obesity, cardiovascular diseases, diabetes, and associated conditions such as the metabolic syndrome and insulin resistance. [14,15]

In T2DM, the role of vitamin D was suggested from the presence of vitamin D receptors (VDR) in the pancreatic β-islet cells. 106 In these cells, the biologically active metabolite of vitamin D (ie, 1,25-dihydroxy-vitamin D; 1,25(OH) 2 D) [16)] enhances insulin production and secretion via its action on the VDR. Indeed, the presence of vitamin D binding protein (DBP), a major predictor of serum levels of 25(OH) D and response to vitamin D supplementation and VDR initiated several studies demonstrating a relationship between single-nucleotide polymorphisms (SNPs) in the genes regulating VDR and DBP and glucose intolerance and insulin secretion. [17]

This further supports a role for vitamin D in T2DM and may explain the reduced overall risk of the disease in subjects who ingest .800 IU/day of vitamin D. [18]

However, an alternative, perhaps related, explanation was recently proposed for the role of vitamin D in the prevention of T2DM based on its potent immunomodulatory functions. 1,25(OH) 2 D modulates the production of the immunostimulatory IL-12 and the immunosuppressive IL-10, and VDRs are present in most types of immune cells. In this respect, supplementation with vitamin D or its bioactive form, 1,25(OH)2D, [19] improved insulin sensitivity by preventing the excessive synthesis of inflammatory cytokines. This effect of vitamin D on cytokine synthesis is due to its interaction with vitamin D response elements present in the promoter region of cytokine-encoding genes. This interaction down regulates the transcriptional activities of cytokine genes and attenuates the synthesis of the corresponding proteins. Vitamin D also deactivates NFκB, which transcriptionally regulates the proinflammatory cytokine-encoding genes. Further, the Correlation

analysis showed significant association of Vitamin D with inflammatory markers in newly diagnosed type 2 diabetics. Increased levels of hsCRP have been significantly associated with unfavorable outcomes. [20]

Decreased insulin sensitivity may lead to enhanced hsCRP expression by counteracting the physiological effects of insulin on hepatic acute-phase protein synthesis. [21]

We observed significant increase in hsCRP levels of newly diagnosed type 2 diabetic group when compared with that of control group. In addition to being a marker of disease presence, hsCRP has been found to bind to endothelial cell receptors promoting apoptosis, and it has been shown to co localize with oxidized LDL in atherosclerotic plaques. hsCRP also stimulates endothelial production of pro-coagulant tissue factor, leukocyte adhesion molecules, and chemotactic substances and inhibits endothelial cell nitric oxide (NO) synthase (eNOS), resulting in abnormalities in the regulation of vascular tone. [22]

hsCRP has been demonstrated to increase the expression of ICAM-1, VCAM-1, and MCP-1 in a concentration-dependent fashion. [23]

Likewise, hsCRP has been demonstrated to facilitate native LDL uptake into macrophages, an important step in foam-cell formation. [24]

Hyperglycemia acutely increases circulating cytokine concentrations. HDL-cholesterol down regulates expression of adhesive molecules on the surface of vascular endothelium and inhibits platelet aggregation and thus has anti-inflammatory and antithrombotic properties. [25]

Our results show that the endothelial dysfunction markers VCAM-1, ICAM-1 levels were higher in diabetic patients than healthy group, which are quite similar to other studies in diabetes [26] and cardiovascular disease. [27]

The adhesion molecule VCAM -1, ICAM-1 are established markers for endothelial dysfunction and they represent major receptors controlling the influx of monocytes and other inflammatory cells into the arterial wall, their expression is considered as a hallmark in the etiology of atherosclerosis. [28]

In the present study, we also observed that serum IL-1β concentrations were significantly higher in cases than in controls. Proinflammatory cytokines secreted by adipose tissue and the other tissues can cause insulin dysfunction in adipose tissue, skeletal muscle and liver by inhibiting insulin signal transduction. Accumulating evidence indicates that diseases related to metabolic syndrome are characterized by abnormal cytokine production, including elevated circulating IL-1β, increased acute-phase proteins, e.g., CRP and activation of inflammatory signaling pathways. [29]

IL-1β plays an important role in lipid metabolism by regulating insulin levels and lipase activity under physiological conditions. Previous studies have described a positive association between IL-1β gene polymorphism and obesity, suggesting functional effects on fat mass, fat metabolism and body mass. [30]

Recent evidence has shown that IL-1β plays a role in various diseases, including autoimmune diseases such as inflammatory bowel diseases and type 1 diabetes, rheumatoid arthritis, as well as in diseases associated with metabolic syndrome such as atherosclerosis, chronic heart failure and type 2 diabetes. [31]
IL-1β production and secretion from pancreatic islets have also been reported. [32]

V. Conclusion

From this study, it is concluded that lower levels of vitamin D is associated with increased inflammatory markers. Therapeutic interventions to increase the vitamin D levels and reduce the inflammation should be included as a part of treatment in newly diagnosed type diabetics.

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