Vitamin D levels and tumor necrosis factor-α activity in newly diagnosed patients with type 2 diabetes mellitus: An exploratory study in North-East India

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ABSTRACT

Background: Diabetes poses a global threat, leading to hospitalizations and premature death if not managed properly. It involves tumor necrosis factor-α (TNF-α), an inflammatory cytokine, which binds to TNF-α receptor-1, triggering sphingomyelinase, and ceramide production. Vitamin D acts as an anti-inflammatory agent, reducing inflammatory cytokines and proinflammatory cell growth, and benefiting diabetes mellitus (DM). **Aims and Objectives:** The primary objective was to compare serum Vitamin D and TNF-α levels in diabetics and non-diabetics. The secondary objective was to explore Vitamin D and TNF-α correlation in newly diagnosed type 2 diabetes. **Materials and Methods:** A case–control study involved 92 subjects in each group. Data included demographics, clinical assessments, glycemic parameters, Vitamin D, and TNF-α. Statistical analysis used student's t-test and Pearson correlation (P<0.05). **Results:** The cohort comprised 34 females (36.96%) and 58 males (63.04%). Cases had significantly higher glycemic levels: FBG (247.2±53.92 mg/dL vs. 91.45±16.64 mg/dL), postprandial plasma glucose (319.7±72.08 mg/dL vs. 111.3±21.76 mg/dL), and glycated hemoglobin (HbA1c) (10.39±2.41% vs. 5.43±0.48%). Mean serum Vitamin D in cases (22.63 ng/mL) was significantly lower than controls (58.15 ng/mL), and mean TNF-α in cases (5.01 pg/mL) was higher than controls (4.63 pg/mL, P<0.0001). HbA1c negatively correlated with serum Vitamin D (r = − 0.7461) and positively with TNF-α (r = 0.7831). Vitamin D correlated negatively with TNF-α (r = − 0.6481). **Conclusion:** The study revealed lower serum Vitamin D and higher TNF-α levels in cases, with a significant inverse association. It also found an inverse link between Vitamin D and HbA1c and a strong positive correlation between HbA1c and TNF-α. Recognizing Vitamin D and TNF-α's diagnostic significance promises innovative strategies for managing type 2 DM.

Key words: Newly diagnosed type 2 diabetes mellitus; Glycemic parameters; Vitamin D; Tumor necrosis factor-α

INTRODUCTION

Diabetes mellitus (DM) is a pressing global health concern that affects individuals irrespective of socioeconomic status or geographical boundaries. Poorly managed diabetes can lead to severe complications, frequent hospitalizations, and premature deaths. The prevalence of DM has surged significantly over the past two decades, with the global count reaching 415 million cases in 2017, up from approximately 30 million cases in 1985.[1] Notably, India

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has experienced a substantial rise in diabetes cases, with over 77 million reported cases by March 2020 and an alarming prevalence rate of 8.9%.[2] Disturbingly, projections indicate a continued increase, with an estimated 1.3 billion individuals in India likely to be affected by diabetes by 2045.[3] Moreover, the North-East region of India has been identified as having an exceptionally high prevalence of diabetes, reaching 19.78%.[4]

DM is characterized by hyperglycemia and represents a chronic inflammatory condition. During its progression, an inflammatory cytokine called tumor necrosis factor-α (TNF-α) is notably produced, primarily synthesized in adipocytes and peripheral tissues. TNF-α activates sphingomyelinase, leading to the production of ceramide, which interferes with insulin signaling and glucose uptake, contributing to insulin resistance.[5] In addition, TNF-α induces Fas expression in pancreatic islets, leading to the apoptosis of pancreatic β-cells.

Vitamin D, aside from its role in maintaining calcium homeostasis, exhibits significant anti-inflammatory properties, regulating inflammatory cytokine production and inhibiting proinflammatory cell proliferation. These anti-inflammatory effects may be beneficial in the context of DM. Moreover, Vitamin D has been associated with enhancing insulin sensitivity through various mechanisms, such as stimulating the expression of insulin receptors (IR) and activating transcription factors involved in fatty acid metabolism in insulin-responsive tissues.[6]

Given the link between inflammation, TNF-α and insulin sensitivity, and the potential role of Vitamin D in mitigating inflammation and enhancing insulin sensitivity, our study aims to explore the following research question: "Is the decreased level of Vitamin D associated with increased activity of TNF-α among newly diagnosed patients with type 2 DM?"

This exploratory study seeks to contribute to the understanding of the relationship between Vitamin D levels and TNF-α activity in individuals with newly diagnosed type 2 DM, particularly in the context of the higher prevalence of diabetes in North-East India. The findings from this research could shed light on potential therapeutic targets and preventive measures for diabetes management in this population.

Aims and objectives
The primary objective is to estimate the levels of Vitamin D and TNF-α in these patients. In addition, the study aims to achieve two secondary objectives: (i) To compare the serum levels of Vitamin D and TNF-α between individuals with diabetes and non-diabetic individuals and (ii) to determine the correlation between Vitamin D and TNF-α in newly diagnosed cases of type 2 DM.

MATERIALS AND METHODS

Study design and participants
This study was designed as a hospital-based case–control study conducted in the department of biochemistry over a period of 1 year from July 2020 to June 2021. The study protocol received approval from the Institutional Ethics Committee (reference ID: SMEJ/JMCH/MEU/841/pt-1/2011/5333(A) dated June 17, 2020). The study followed good clinical practice guidelines, and participation was voluntary without any reward or incentive. Informed written consent was obtained from each participant, ensuring the anonymity of collected data. The inclusion criteria consisted of patients aged 30 years or older with newly diagnosed type 2 DM based on the diagnostic criteria provided by the American Diabetes Association 2020. These criteria included fasting plasma glucose (FPG) level >126 mg/dL (7.0 mmol/L), postprandial plasma glucose (PPPG) level ≥200 mg/dL (11.1 mmol/L), glycated hemoglobin (HbA1C) ≥6.5% (48 mmol/mol), or random plasma glucose level >200 mg/dL (11.1 mmol/L).[7] Controls were selected from apparently healthy individuals of matching age and sex, who were attendants of non-diabetic patients and had no history of diabetes among their close relatives. Exclusion criteria comprised known hemoglobin opathies, anemia, G-6-PD deficiency, acute blood loss, recent blood transfusion history, renal failure, pancreatitis, liver disease, alcoholism, chronic obstructive pulmonary disease, and refusal for participation.

Data collection
After explaining the study's objectives and obtaining written consent, participants underwent interviews and clinical examinations. The collected sociodemographic information included age, gender, educational background, marital status, medical and medication history, family history, and any instances of substance abuse. Clinical examination results were thoroughly documented. Intravenous blood samples were collected following aseptic and sterile procedures. The blood samples were processed to measure fasting and PPPG levels, HbA1C, Vitamin D, and TNF-α.

Laboratory methods
FPG and PPPG were assessed using the colorimetric estimation method employing glucose oxidase-peroxidase on the VITROS 5600 analyzer.[8] HbA1C levels were determined through cation-exchange high-performance liquid chromatography on the D-10 instrument. Serum Vitamin D levels were measured using a competitive

immunoassay method on the VITROS 5600 analyzer.[9] Serum TNF-α levels were quantified using the Sandwich enzyme-linked immunosorbent assay method.[10]

Sample size estimation and sampling method
The sample size was calculated as 92 in each group (Cases and control) using Epi Info software with a 95% confidence interval, 80% power, and a prevalence of 19.78%. Both cases and controls were recruited through purposive sampling, adhering to the specified inclusion and exclusion criteria.

Statistical analysis
Statistical analysis was conducted using MS Excel and GraphPad Prism 9.0.0. The variables were analyzed using the student's t-test and Pearson correlation coefficient to determine any differences. $P < 0.05$ was considered statistically significant.

RESULTS

Participants and demographic characteristics
A total of 92 newly diagnosed type 2 DM patients and 92 age- and sex-matched healthy controls were included in the study. Among the participants (n=34) in both case and control groups, 36.96% were female, while (n=58) 63.04% were male (Figure 1). The average age of female participants in the case group was 49.47 years, while male participants had an average age of 46.72 years. In the control group, the average age of female participants was 48.71 years, and male participants had an average age of 45.31 years.

Glycemic control status
The study recorded FPG levels in the case group as 247.2±53.92 mg/dL and in the control group as 91.45±16.64 mg/dL. Similarly, PPPG levels in cases were 319.7±72.08 mg/dL, while, in the control group, it was 111.3±21.76 mg/dL. HbA1c levels in the case group were 10.39±2.41%, and in the control group, they were 5.43±0.48%.
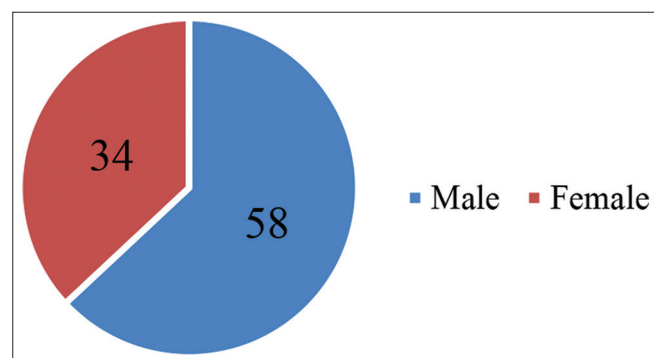

Figure 1: The demographic characteristics of the study population

Vitamin D and TNF-α status
In the case group, the serum level of Vitamin D was measured to be 22.63±5.12 ng/mL, while in the control group, it was 58.15±17.93 ng/mL. The serum level of TNF-α in cases was 5.01±0.63 pg/mL, compared to 4.63±0.57 pg/mL in the control group. The specific values for FPG, PPPG, HbA1c, Vitamin D, and TNF-α are provided in Table 1, and their graphical representations are depicted in Figure 2a and b.

Correlation study analysis
The correlation study of cases using Pearson Correlation coefficient revealed a negative correlation between HbA1c and serum Vitamin D (r=−0.7461) and a positive correlation between HbA1c and serum TNF-α (r=0.7831). Furthermore, when Vitamin D was compared with TNF-α, it showed a negative correlation with r=−0.6481 (depicted in Figure 3a-c).

DISCUSSION

In spite of the advancements made in medical sciences, type 2 DM remains a grave concern for our society. This fact is evident due to the remarkable surge in its prevalence over the past two decades. The seriousness of this condition becomes apparent when considering its global prevalence, which rose from 30 million cases in 1985 to a staggering 415 million cases in 2017.[1] Hence, it is imperative to meticulously scrutinize even the most minute aspects of this disease.

In this particular study, our objective was to explore whether there exists any correlation between the serum level of Vitamin D and TNF-α in newly diagnosed cases of type 2 DM. Conducting a case–control study, we observed a predominant occurrence of this disease in males (63.03%). Notably, a comparable pattern was also evident in studies on Indian population by Pradeepa et al., and Ranasinghe et al.[11,12]

When assessing glycemic control, we observed that all parameters, namely, FPG, PPPG, and HbA1c, were significantly elevated in the cases compared to the control group (P<0.0001). Furthermore, our study discovered a notable difference in the serum levels of Vitamin D between the cases (22.63±5.12 ng/mL) and the control group (58.15±17.93 ng/mL), with the cases exhibiting significantly lower levels. The role of Vitamin D can be explained at various levels, including its impact on insulin synthesis by pancreatic beta-cells and regulation of blood glucose in target cells. The previous studies have demonstrated the presence of the Vitamin D receptor in pancreatic beta-cells and its influence on their function.[13] In

Table 1: The mean, SD, 95% CI and statistical value of FPG, PPPG, HbA1c, Vitamin D, and TNF-α

Parameter	Cases (Mean±SD)	Controls (Mean±SD)	95% CI		Statistical value
			Cases	Controls	
FPG (mg/dL)	247.2±53.92	91.45±16.64	236.0±258.4	88.0±94.89	P≤0.0001 t=26.48
PPPG (mg/dL)	319.7±72.08	111.3±21.76	304.8±334.6	106.8±115.8	P≤0.0001 t=26.54
HbA1c (%)	10.39±2.41	5.43±0.48	9.89±10.89	5.33±5.55	P≤0.0001 t=19.33
Vitamin D (ng/mL)	22.63±5.12	58.15±17.93	21.57±23.69	54.43±61.86	P≤0.0001 t=18.27
TNF-α (pg/mL)	5.01±0.63	4.63±0.57	4.88±5.15	4.51±4.74	P≤0.0001 t=4.37

SD: Standard deviation, FPG: Fasting plasma glucose, PPPG: Post-prandial plasma glucose, HbA1C: Glycosylated hemoglobin, TNF-α Tumor necrosis factor-α
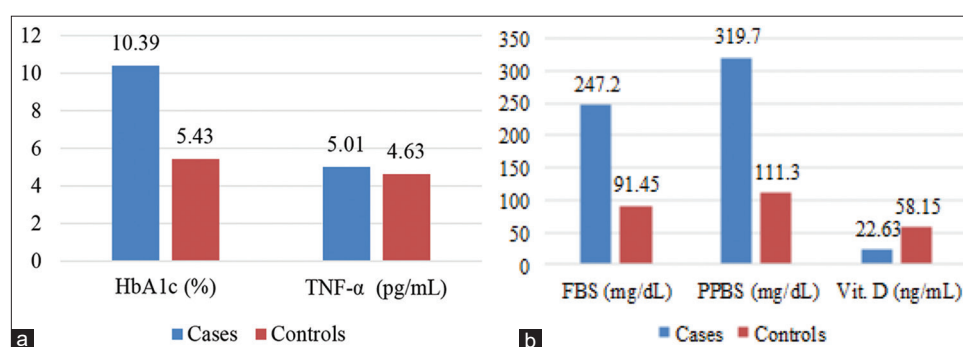

Figure 2: (a) bar diagram showing the mean value of FPG, PPPG and Vit. D, (b) bar diagram showing the mean value of HbA1c and TNF-α. FPG: Fasting plasma glucose, PPPG: Post-prandial plasma glucose, Vit.D: Vitamin - D

addition, the presence of the Vitamin D response element in the promoter region of the IR gene, which regulates IR at the mRNA level, as well as the number of IR and insulin responsiveness for glucose transport, has been observed.[14] The effects of Vitamin D on IR in target cells have also been documented.[15] Consequently, a deficiency in Vitamin D levels can be a key factor contributing to insulin deficiency/resistance in patients with type 2 DM. Multiple studies conducted by Abbasi et al., Kostoglou-Athanassiou et al., Esteghamati et al., Salih et al., Saif-Elnasr et al., and Ahdal and Kumar et al., have consistently identified low levels of Vitamin D in individuals with type 2 DM.[16-22]

Despite both groups having TNF-α serum levels within the normal range (i.e., <8.0 pg/mL), the mean concentration of TNF-α in the cases was significantly higher compared to the mean concentration in controls, with P<0.0001 and a t-value of 4.37.

Insulin signaling heavily relies on the autophosphorylation of the IR and subsequent phosphorylation of IR substrate-1, processes that play a major role in insulin signaling. However, the elevated concentration of TNF-α inhibits these essential steps.[23] In addition, phosphoinositide-3 (PI-3) kinase, an important enzyme in insulin signaling involved in transporting GLUT-4 from intracellular vesicles to the plasma membrane for stimulating glucose uptake from the blood, is also affected by TNF-α. As a result, TNF-α interferes with the activity of PI-3 kinase, leading to decreased glucose uptake from the blood.[24] Numerous studies have demonstrated that an increased level of TNF-α induces insulin resistance both at the hepatic and peripheral levels, resulting in hyperglycemia.[25] Similar trends of higher concentrations of TNF-α in cases compared to controls were observed in studies conducted by Zinman et al., Miyazaki et al., Spranger et al., Swaroop et al., and Alzamil.[26-30]

The serum concentration of Vitamin D showed a negative correlation with HbA1c, as indicated by the Pearson correlation coefficient "r" of −0.7461. This finding is consistent with several other studies that have also reported an inverse relationship between Vitamin D and HbA1c. For example, Kositsawat et al., in 2010, found an inverse correlation between the serum level of Vitamin D and HbA1c in type 2 diabetic patients. Similar results were reported by Zoppini et al., in 2013, Kajbaf et al., in 2014, Buhary et al., in 2017, Alkhatatbeh et al., in 2018, Zhao et al., in 2020, and Hwang et al., in 2022, all of which demonstrated a statistically significant inverse relationship between Vitamin D levels and HbA1c.[31-37]

Moreover, the serum concentration of TNF-α shows a significant positive correlation with HbA1c, as indicated
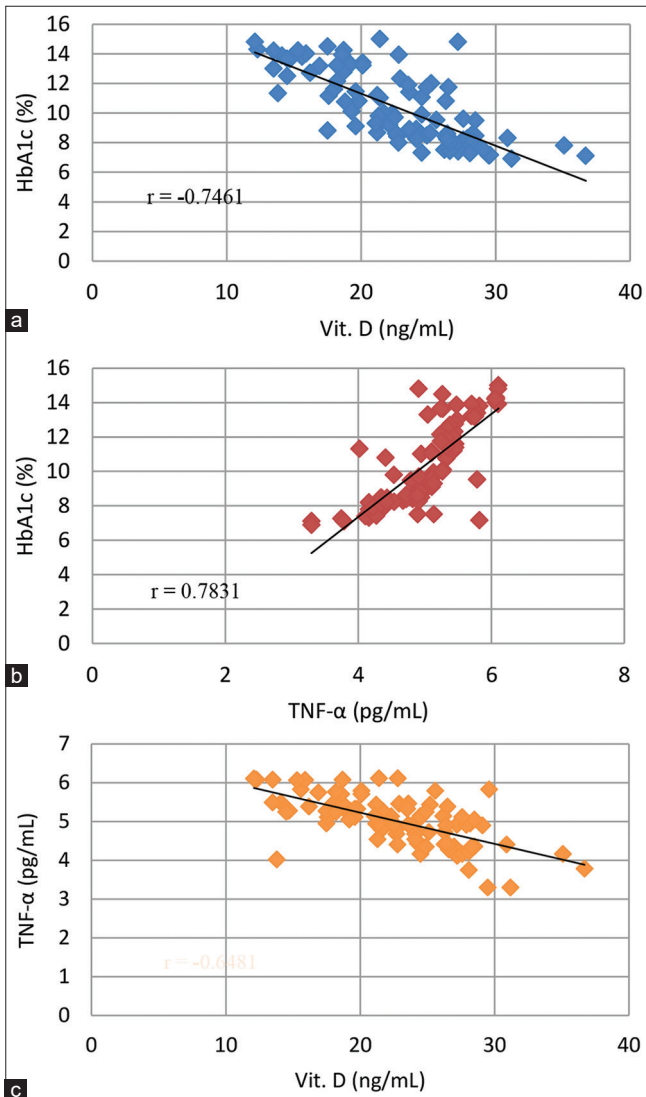
Figure 3: (a) The negative correlation between HbA1c and Vit. D. (b) the negative correlation between HbA1c and TNF-α. (c) The positive correlation between TNF-α and Vit. D. Vit.D: Vitamin – D, HbA1C: Glycosylated hemoglobin

by the Pearson correlation coefficient "r" of 0.7831. This positive relationship between TNF-α and HbA1c was previously observed by Spranger et al., in 2003 and later confirmed by Alzamil, in 2020, further corroborating the findings of the current study.[28,30]

In the present study, when we compared the serum concentration of Vitamin D with TNF-α, a significant negative correlation was observed between the two in the entire study population (including cases and controls). On further examination of the strength of this correlation within the study population, we found that the negative correlation between vitamin D and TNF-α was stronger in cases (r=−0.6481) compared to controls (r=−0.4369). This difference may be attributed to the decreased concentration of serum Vitamin D in cases, which consequently leads

to a reduction in the anti-inflammatory response in these individuals, resulting in an increase in TNF-α levels.

DM is characterized as a low-grade chronic inflammatory condition, and similar to other inflammatory conditions, it may be associated with elevated proinflammatory cytokines such as TNF-α. The anti-inflammatory properties of Vitamin D have been well-established in previous research. Therefore, it can be assumed that when the serum level of Vitamin D decreases, its anti-inflammatory effects are reduced, ultimately leading to an increase in the serum level of TNF-α.

Limitations of the study
The present study has few limitations. The cross-sectional nature of the study helps in determining correlations, but limits establishing the casuation. Also one time data collection reflects its snap shot nature, thus challenging the derivation of temporal sequences. The data was self reported, hence has the potential of recall bias. Inclusion of patients from single centre limits its generalization to entire population which has variations in culture, lifestyle and access to healthcare. Duration of one year is inadequate to analyze long term trends, which can be addressed by prospective studies of longer duration and regular follow of the patients.

CONCLUSION

DM remains a significant health concern in modern society. However, early detection and proper treatment can prevent its complications. The study findings revealed that newly diagnosed cases of type 2 DM exhibited significantly lower serum Vitamin D levels and higher TNF-α levels compared to the control group. Moreover, a statistically significant inverse relationship was observed between these two parameters. In addition, there was a negative correlation between Vitamin D and HbA1c levels, while a strong positive correlation was found between HbA1c and TNFα levels.

These findings underscore the importance of considering Vitamin D and TNF-α as essential diagnostic parameters in the management of type 2 DM. By recognizing the significance of these biomarkers, we open up promising possibilities for developing novel and improved strategies for effectively managing and treating this complex metabolic disorder. Early intervention based on the assessment of Vitamin D and TNF-α levels could potentially aid in better disease monitoring and tailored treatment approaches, ultimately leading to improved outcomes and a better quality of life for individuals affected by type 2 DM. Further, research and clinical trials are warranted to validate

and expand on the implications of these findings, paving the way for more targeted and personalized therapeutic interventions in the future.

ACKNOWLEDGMENT

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