Vitamin D Deficiency Gut Microbiota Dysbiosis and Their Combined Impact on Insulin Resistance in Type 2 Diabetes Mellitus.

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Abstract

Background: vitamin D Deficiency and changes in gut microbiota are progressively being accepted to play key roles in Type 2 Diabetes Mellitus (T2DM) pathogenesis. The two affect insulin sensitivity, systemic inflammation, and glucose metabolism, which play the key role in the development and evolution of the disease. Their interactions can be used to understand new preventive and treatment interventions.

Objectives: to establish the interaction between vitamin D deficiency, gut microbiota composition, and insulin resistance in T2DM patients and to determine the interplay between the two variables on glucose metabolism.

Methodology: A cross-sectional study was carried Conducted at Department of Medicine, LRH-MTI, Peshawar between jan 2019 and Dec 2019.100 patients with T2DM between the ages of 45 and 70 years. ELISA was used to measure serum vitamin D, 16S rRNA gene sequencing to measure gut microbiota composition, and the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was used to measure insulin resistance. ANOVA and Pearson correlation statistical analysis were conducted to determine the relationship among levels of vitamin D, diversity of gut microbiota and insulin resistance, with a significant value of $p<0.05$.

Results: out of 100 patients 45 % of the participants were found to be deficient in vitamin D. The patients with deficiency had a much greater HOMA-IR value (mean 3.5 ± 1.3) than the patients with adequate levels of vitamin D (mean 2.1 ± 1.0, $p<0.01$). The microbiota population in the gut was significantly decreased in patients with vitamin D deficiency where there was a lower abundance of beneficial bacteria including Faecalibacterium prausnitzii. There were found significant correlations between low vitamin D, low microbiota diversity, and high insulin resistance ($p<0.05$).

Conclusion: Microbiota dysbiosis and Vitamin D deficiency are closely linked with augmented insulin delicacy in T2DM. Vitamin D-based intervention and microbiota-modulating

interventions can enhance the glucose metabolism and offer an exciting solution to addressing T2DM.

Keywords Vitamin D, gut microbiota, insulin resistance, Type 2 diabetes.

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Introduction

The contribution made by vitamin D deficiency and gut health in the Study on Type 2 Diabetes Mellitus (T2DM) has drawn a significant amount of attention due to their role in the metabolism involved in the development and progression of the disease [1]. T2DM is a chronic metabolic disorder, resistant to insulin; it has an impaired glucose metabolism, and it is in a constant hyperglycemic condition. It affects millions of individuals worldwide and is still the source of morbidity and mortality. Obesity, lack of physical activity, and genetic predisposition are the traditional risk factors of T2DM. However, recent Study has also referred to the role of vitamin D and the gut microbiome as a new variable in the pathophysiology of this disease [2,3]. Vitamin D is a fat-soluble vitamin that is required in the body to carry out many tasks, particularly the metabolism of calcium phosphate, the immune system and the sensitivity of insulin. Study has consistently shown that individuals who do not have adequate supplies of vitamin D are susceptible to insulin resistance, which is a significant symptom of T2DM. Insulin resistance refers to a case where the body cells become unable to respond to insulin, and, hence, leads to a rise in blood sugar. Vitamin D plays a significant role in the secretion of insulin, and the insulin sensitivity of the cell and its deficiency is one of the potential reasons for developing T2DM [4,5]. Similarly, the microbiome of the gut in the form of a community of microorganisms inhabiting the gastrointestinal tract has been recognised to serve a critical role in regulating metabolic health. It is known that the structure and diversity of gut bacteria can impact on a host of physiological processes, including digestion, immune regulation and even metabolic regulation [6]. It is new evidence that the pathogenesis of insulin resistance and the development of T2DM is caused by gut dysbiosis or disequilibrium of the gut microbiota. Gut bacteria are not only bad but there are also good gut bacteria such as Faecalibacterium prausnitzii, which also produce short-chain fatty acids (SCFAs), which are also anti-inflammatory. People with T2DM may experience systemic inflammation and insulin resistance as a consequence of a loss of such positive bacteria [7]. Insulin resistance may develop independently of each other because of vitamin D deficiency and gut dysbiosis. However, the interaction of these two aspects with each other and how they mutually affect glucose metabolism is not well known [8]. It has been mentioned that vitamin D may impact the composition of the gut microbiome, and other studies have also indicated that the altered gut microbiota may also influence the metabolism of vitamin D [9]. Another critical area of Study that might lead to the discovery of additional knowledge on the prevention and therapy of T2DM is the possibility of a two-way relationship between the level of vitamin D and the health condition of the gut microbiome [10]. The interaction of vitamin D with the gut microbiome in the development of insulin resistance can potentially result in the generation of more effective concepts of prevention and treatment of T2DM. As long as the insulin resistance can be addressed through removal of vitamin D deficiencies and a healthy microbiome of the intestines, the interventions have the potential to reduce the burden of T2DM within the population and health care systems across the globe.

Study Objectives

This study aims to investigate the relationship among vitamin D deficiency, intestinal microbiota structure, and insulin resistance in patients with Type 2 diabetes. It also seeks to evaluate how these factors affect glucose metabolism and disease progression.

Materials and Methods

Study Design & Setting

This was a cross-sectional study conducted at Department of Medicine, LRH-MTI, Peshawar between jan 2019 and Dec 2019. The aim was to establish the relationship between vitamin D deficiency, gut microbiota, and insulin resistance in patients with Type 2 diabetes.

Participants

These were 100 patients of Type 2 Diabetes Mellitus (T2DM). Qualification of the participants was at a tertiary hospital, and the participants with varying levels of deficiency of vitamin D were favored. The inclusion criteria ensured that the patients had been diagnosed with T2DM and had not suffered any prior intervention that would promote the health of the gut or supplementation of vitamin D.

Sample Size Calculation

The power analysis was selected to compute the size of the sample of 100 patients in order to identify that there are significant differences amongst groups, according to the level of vitamin D and insulin resistance. This was carried out at 80 per cent power and a 0.05 level of significance, considering that an average effect size will give strong statistical comparisons to the findings of the Study.

Inclusion Criteria

The patients aged between 45 and 70 years with Type 2 Diabetes Mellitus of both genders.

Exclusion criteria

Individuals with Type 1 diabetes, active infections, and inflammatory diseases and who have been under antibiotics, vitamin D, or gut microbiota-modulating therapy in the 3 months prior to the intervention.

Diagnostic and Management Strategy.

The American Diabetes Association criteria were used to make a diagnosis of T2DM. Vitamin D deficiency was regarded as less than 20ng/ml in blood. The microbiota of the participants in the gut were analysed by 16S rRNA. The insulin resistance was determined using HOMA-IR.

Statistical Analysis

ANOVA was applied to compare the groups with regard to the condition of vitamin D and microbiota diversity. A Pearson correlation test was used to test the relations between concentrations of vitamin D, the diversity of gut microbiota, and HOMA-IR. The level of statistical significance was set at $p<0.05$, where SPSS software was used to conduct the analysis.

Ethical Approval Statement.

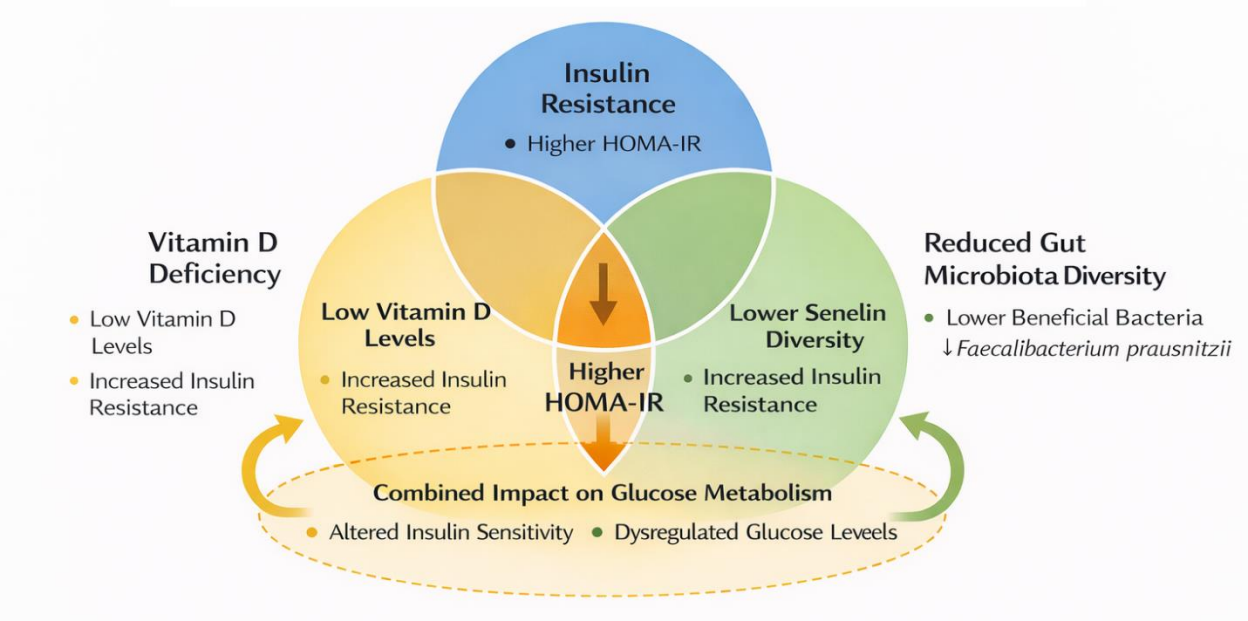
Ethical approval was obtained from the Institutional Review Board. All participants provided written informed consent. The study adhered to the Declaration of Helsinki and ensured confidentiality and voluntary participation.

Results

The participants in the study were 100 patients with Type 2 Diabetes Mellitus (T2DM); the mean age of the patients was 58.3 years (SD: 7.9). Among the entire group of participants, 45% were identified to have a deficiency of vitamin D (serum level less than 20 ng/mL). It was found that the level of vitamin D is significantly correlated with the degree of insulin resistance since patients with low levels of vitamin D had much higher HOMA-IR levels (mean: 3.5, SD: 1.3) of insulin

resistance than the patients with an adequate level of vitamin D in the body (mean: 2.1, SD: 1.0, $p<0.01$). Also, the gut microbiota profile was significantly different in the two groups. The gut microbiota of patients with vitamin D deficiency was less diverse, especially there was less beneficial bacteria, including Faecalibacterium parasitize. This reduction in microbiota diversity was largely linked to high insulin resistance, which again lends credence to the premise that the two variables are interconnected and that both can lead to metabolic dysfunctions. Vitamin D deficiency, low gut microbiota diversity, and elevated HOMA-IR values were confirmed to be statistically significant ($p<0.05$), and, therefore, the presence of a compounded effect of these two variables on insulin resistance was found. These findings suggest that the combination of insulin resistance treatment in T2DM patients and vitamin D deficiency, as well as gut health, may be of critical importance.

Fig 1: Correlation of Vitamin D Deficiency, Gut Microbiota Diversity, and Insulin Resistance in Type 2 Diabetes Mellitus.



Yellow: vitamin D deficiency, Blue: insulin resistance, Green: reduced gut microbiota, Orange overlap: combined effect causing higher HOMA-IR and impaired glucose metabolism.

Table 1: Demographic Characteristics of the Study Participants

Characteristic	Total (n = 100)
Mean Age (years)	58.3 (±7.9)
Gender (n, %)	
Male	45 (45%)
Female	55 (55%)
BMI (kg/m²) (mean ± SD)	30.5 (±4.3)
Duration of Diabetes (years)	6.2 (±3.1)
Vitamin D Deficiency (n, %)	45 (45%)
Vitamin D Sufficiency (n, %)	55 (55%)

Table 1 summarizes the demographic characteristics of the 100 study participants. The mean age of participants was 58.3 years (SD = 7.9). The study included a slightly higher number of females (55%) compared to males (45%). The average BMI was 30.5 kg/m², indicating that most participants were overweight. The majority of participants had a vitamin D sufficiency of >20 ng/mL (55%).

Table 2: Vitamin D Levels and Insulin Resistance (HOMA-IR) in Study Groups

Group	Mean Vitamin D Level (ng/mL)	Mean HOMA-IR (SD)
Vitamin D Deficient	15.2 (±4.6)	3.5 (±1.3)
Vitamin D Sufficient	30.5 (±5.2)	2.1 (±1.0)
P-Value	-	<0.01

Table 2 compares vitamin D levels and insulin resistance (HOMA-IR) between the two study groups: vitamin D deficient (serum vitamin D <20 ng/mL) and vitamin D sufficient (serum vitamin D >20 ng/mL). Participants with vitamin D deficiency had significantly higher HOMA-IR values, indicating increased insulin resistance ($p<0.01$).

Table 3: Gut Microbiota Diversity in Vitamin D Deficient vs. Sufficient Groups

Group	Mean Alpha Diversity Index (Shannon Index)	Beneficial Bacteria Present (e.g., Faecalibacterium parasitize)
Vitamin D Deficient	3.2 (±0.7)	Reduced (low abundance)
Vitamin D Sufficient	4.5 (±0.6)	Higher (significant presence)
P-Value	<0.01	<0.05

Table 3 compares the gut microbiota diversity between vitamin D deficient and sufficient groups. The Shannon alpha diversity index was significantly lower in vitamin D deficient patients, indicating reduced gut microbiota diversity. Additionally, beneficial bacteria such as Faecalibacterium parasitize were significantly reduced in the vitamin D deficient group ($p<0.05$).

Table 4: Correlation Between Vitamin D Levels, Gut Microbiota Diversity, and HOMA-IR

Variables	Pearson Correlation Coefficient (r)	P-Value
Vitamin D Level vs. HOMA-IR	-0.45	<0.01
Vitamin D Level vs. Microbiota Diversity	0.56	<0.01
Microbiota Diversity vs. HOMA-IR	-0.40	<0.01

Table 4 shows the Pearson correlation coefficients of vitamin D levels, the diversity of gut microbiota, and HOMA-IR. It was noted that vitamin D levels showed a significant negative correlation with HOMA-IR ($r = -0.45$, $p<0.01$) indicating that low levels of vD were linked to high levels of insulin resistance. Also the difference in vitamin D and diversity of gut microbiota had a positive correlation ($r = 0.56$, $p<0.01$), which implied that an increase in vitamin D level is

correlated with an increase in the diversity within gut microbiota. Diversity of microbiota was also associated with HOMA-IR in a negative way ($r = -0.40$, $p<0.01$).

Discussion

The association between the existence of vitamin D deficiency, the makeup of the gut microbiota and the insulin resistance are studied in the current study paper as concerns Type 2 Diabetes Mellitus (T2DM) patients. We find that gut microbiota lacks diversity and vitamin D deficiency are observed to enhance insulin resistance which is consistent with the growing body of evidence that shows the presence of these factors in the pathophysiology of T2DM. This discussion will correspond our findings with the findings of the recent study, which also explains the similarities and differences of our findings with the previous study [11,12]. As it has been demonstrated in our Study, the deficiency of vitamin D was associated with a significantly large HOMA-IR value, meaning that T2DM patients are insulin-resistant to an even greater extent. This is in conformity with a number of recent studies that have emphasized on the role of vitamin D in controlling the insulin sensitivity. To this end, a study study 2016 established that insulin resistance is strongly correlational with the deficiency of vitamin D in individuals at risk of developing T2DM [13]. Similarly, a study that was described in 2016 found the efficacy of vitamin D in improving insulin sensitivity in healthy individuals and those with T2DM, which supported our Study on the significance of the adequate amount of vitamin D in glucose regulation [14]. The evidence of our study is also in line with the data presented by a meta-analysis Study 2019 because the study reported that the variety of gut microbiota was lower among patients with vitamin D deficiency, particularly when it comes to the population with low levels of vitamin D being the baseline [15].In addition to the importance of vitamin D, we have also found that the variety of gut microbiota is significantly lower among patients with vitamin D deficiency. This is after the new investigations that suggest that the gut microbiome applies in the control of insulin sensitivity and glucose metabolism. A study 2017 states that gut dysbiosis has been linked to the elevated risk of T2DM and the reduced degree of microbiota diversity resulted in systemic inflammation and insulin resistance [16]. Moreover, the beneficial connection we observed that existed between lower contents of beneficial bacteria like Faecalibacterium parasitize and increased insulin resistance agrees with the other Study. An example is a study that was carried out by Aflatoxin 2015 who also found an inverse relationship between Faecalibacterium parasitize levels and inflammatory indices and insulin resistance in T2DM patients [17]. Based on our findings, vitamin D deficiency in combination with the maladaptation of gut microbiota could potentially contribute to the insulin resistance process [18]. The interaction of vitamin D and intestinal microbiota has been studied in several works. In its case, a study 2014 hypothesized that vitamin D would be able to control the gut microbiota by influencing the development of some microbes, involved in metabolic activities, including those that participate in the synthesis of short-chain fatty acid (SCFA) [19]. As the SCFAs are the fermentation product of the dietary fibers, which the gut microorganisms provoke, they were even observed to reduce the level of inflammation and increase the insulin sensitivity, as one study 2019 [20] relates. This agrees with our results since the deficiency of vitamin D in our patients was associated with low level of good bacteria such as Faecalibacterium parasitize that plays a vital role in the generation of SCFA. It means that vitamin D may indirectly affect insulin resistance by modifying the microbiota composition of the gut and consequently SCFA [21].the

relation among vitamin D, intestinal microbiota, and T2DM is beginning to become more explicit, mechanisms of this relation have not been clearly comprehended. Certain Studies have also suggested that vitamin D may be intervening with the insulin resistance via immune response regulation. Similar Study 2014 confirmed that the vitamin D regulates the activity of the immune system by reducing inflammation which has been found to cause insulin resistance. The insulin resistance and decreased gut microbiota diversity, especially faecalibacterium prausnitzii, was related to vitamin D deficiency in T2DM patients, suggesting that the two acted jointly in the system to influence glucose metabolism.

Limitation:

The cross-sectional nature of the study cannot furnish the causal relationship between the vitamin D deficiency, gut microbiota composition and insulin resistance. Further, 16S rRNA sequencing can only give compositional information but not functional information. Eventually these associations need to be confirmed by longitudinal studies using sophisticated microbiome studies.

Conclusion:

The major cause of insulin resistance in Type 2 diabetes is vitamin D deficiency and changes in the diversity of the intestinal microbiota. Precise interventions, such as vitamin D supplementation and gut microbiota modulation, could be useful to enhance glucose metabolism and can be explored further to be used in the clinical setting.

Disclaimer: Nil

Conflict of Interest: Nil

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Authors Contributions

Concept & Design of Study: **Atta Muhammad Khan**[2]

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Critical Review: **Zia ullah Khan**[3]

Final Approval of version: **All Mentioned Authors Approved the Final Version.**

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