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Glycosylation Gap in Type II Diabetes Mellitus Patients with Vitamin D Deficiency

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ABSTRACT

Diabetes mellitus represents a major health problem worldwide. DMis usually associated with hypovitaminosis D. Glycosylation is a non-enzymatic process associated with hyperglycemia. This work aims to explore the changes in glycosylation gap and VDR gene expression in type II DM patients with different vitamin D (vit. D) status in Mosul Province. This study recruit 300 subjects visiting Orkida's private laboratory for general screening tests. From May 2020 to August 2021, Only 80 of them fit the inclusion criteria of this work. They were divided into four groups of twenty each Serum fructosamine assay using the NBT-spectrophotometric methods12 and Vit D level estimation by using VIDAS® 25 OH Vitamin D Total - BIOMERIEUX using the Enzyme-Linked Fluorescent Assay (ELF) technique. Cells used for VDR qPCR analysis. The results of this work shows significant reduction in vitamin D levels between the three tested groups ($p<0.05$) in comparison to control. The glycosylation gap showed a significant elevated in all groups in comparison to the control-group ($p<0.01$). Significant reduction was noticed in VDR expression in all groups in comparison to the control-group was noticed. VDR expression significantly reduced with elevation in glycosylation gap in both normoglycemic with low Vit D and type II diabetic with sufficient and insufficient vit D group.

Keywords: diabetes, glycosylation gap, Fructosamine, VDR expression.

Introduction

Diabetes mellitus (DM) and its complications represents a major health issue worldwide. Diabetic patients exhibit a variety of metabolic changes associated with hyperglycemia, which are caused by either a lack of very low insulin levels, faulty insulin, or both(1). Type II DM patients make up to 95% of all diabetic patients, with a prevalence of 25 to 33%, and it is expected to increase by the next decay(2). The process of glycosylation is a non-enzymatic process that related to prolonged hyperglycemia conditions. Glycosylation affects various protein types, causing changes in their properties and function(3); the best examples were HbA1c and fructosamine, where glucose binds to hemoglobin and albumin, respectively. These glycosylation products have been used for a long time as biomarkers for.

Vitamin D (vit D) is known to have hormone-like effects that directly relate to regulating insulin action and sensitivity as well as some aspects of glucose metabolism via a variety of mechanisms such as controlling-cell insulin secretion, regulating calbindin function, regulating calcium fluxes, increasing insulin

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receptors in muscles and adipose tissue, and increasing peroxisome proliferator-activated receptor-delta (PPAR) activation in muscle and adipose tissue, which improves (4)(5).

Also, vit D significantly affects mitochondrial integrity and respiration, leading to increased oxidative phosphorylation in tissues(6), reducing reactive oxygen spices (ROS) formation and reducing cell apoptosis(7). Despite the sunny weather and high fatty diet, many studies show that the Middle Eastern population has very low levels of serum 25-hydroxyvitamin-D(8).The prevalence in this region may reach up to 60% of the population, as in Saudi Arabia(9). In Iraq, there is no clear data about the Vit D status of the population and its role in diabetes mellitus. This work tries to changes in glycosylation gap and VDR gene expression in type II DM patients with different Vit D status in Mosul Province.

Methods

Materials

This study recruited 300 subjects visiting Orkida private laboratory for general screening tests. From May 2022 to August 2023, Only 80 of them fit the inclusion criteria of this work. Subjects divided into four groups of twenty each, as follows: Group 1 includes normal, healthy individuals as the control-group. Group two includes normoglycemic subjects with insufficient serum vitamin D according to WHO classification(10). Group 3 includes type II diabetic patients with sufficient Vit D levels. Four type II diabetic patients with insufficient Vit D levels are group together. The studied subjects had age ranged from 31-47 years, with a BMI range of 23-27.5.

This study was approved by scientific committee the department of clinical laboratory sciences'. Seven milliliters of vein blood were collected and divided into 2 ml for HbA1c (11) and 5 ml centrifuged at 3000 rpm for 5 minutes; the serum was used for fructosamine assay using the NBT-spectrophotometric methods(12) and Using the Enzyme-Linked Fluorescent Assay (ELF) method, the estimation of vitamin D level was assessed using VIDAS® 25 OH Vit D Total - BIOMERIEUX - France for the measurement of 25-hydroxyVit D in serum (13).

CATCTTCCTGGATCCTCGCC, TATGAGGGCTCCGAAGGCAC,

TATGAGGGCTCCGAAGGCAC, TATGAGGGCTCCGAAGGCAC,

TATGAGGGCTCCGAAGGCAC, TATGAGGGCTCCGAAGGCAC,

TATGAGGGCTCCGAAGGCA Promega master mix (A6000 USA) was use for amplification. Eco study software used to analyze the results. The data used in this work represented mean SD, using Microsoft Excel 2017 software for statistical analysis. The unpaired t-test used to define the significant changes at p 0.05.

Results and Disscussion

The study's findings demonstrated that there was a significant reduction in vitamin D levels in between the three groups ($p<0.05$) in comparison to the control-group (non-diabetic with sufficient Vit D level) as shown in **Figure 1**.

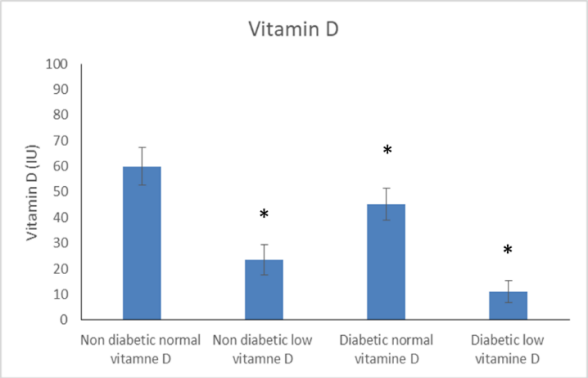

Figure 1. Vitamin D level in Nondiabetic with sufficient Vit D level (Control), non-diabetic with insufficient vitamin D level, diabetic with sufficient Vit D level and diabetic with insufficient Vit D level. * mean significant difference at $p<0.05$.

Glycemic profile results showed a significant elevation in HbA1c in both diabetic groups in comparison to the control-group (p 0.001). In diabetic groups, both fructosamine levels and predicted HbA1c levels were significantly higher than in control-groups (p 0.01). While hypovitamosis normoglycemic subjects showed a significant reduction in fructosamine in comparison to control-group ($p< 0.05$). As shown in **Table 1**, mean blood glucose levels are significantly elevated in the diabetic group only.

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Table 1. Glycemic profile in Nondiabetic with sufficient Vit D level (Control), non-diabetic with insufficient Vit D level, diabetic with sufficient Vit D level and diabetic with insufficient Vit D level. * mean significant difference at p<0.05

Parameter	Non-diabetic normal Vit D Mean ± SD	Non-diabetic low Vit D Mean ± SD	Diabetic normal Vit D Mean ± SD	Diabetic low Vit D Mean ± SD	P- value
Measured HbA1c (%)	5.69± 0.34	6 ± 0.29*	9.24± 1.12*	9.87± 0.45*	0.001
Fructosamine µmol/l	253.8± 12	210± 31*	339± 28*	356± 29*	0.01
Predicted Hba1c (%)	5.42± 0.20	6.18± 0.52*	7.37± 0.49*	7.66± 0.5*	0.01
Mean Blood Glucose (mmol/L)	6.34± 0.59	6.92± 0.51	12.6± 1.95*	15.46± 0.79*	0.01
*Significant difference in comparison to control-group at * p<0.01*					

Glycosylation gap showed significant elevated in all groups in comparison to control-group (on diabetic normal Vit D level) (p<0.01) as shown in **Figure 2**.

As shown in **Figure 2**, the glycosylation gap was significantly higher in all groups compared to the control-group (on diabetic normal Vit D level) (p 0.01).

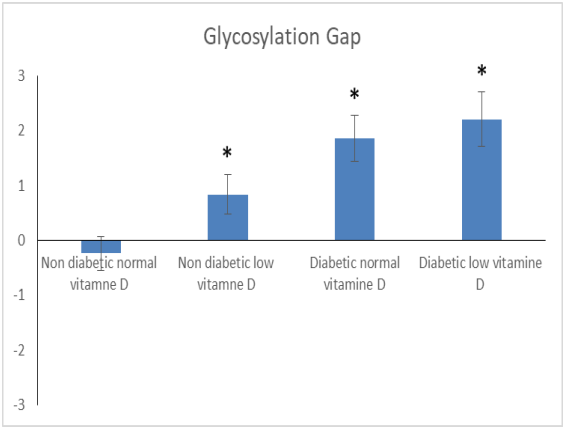

Figure 2. Glycosylation Gap in Nondiabetic with sufficient Vit D level (Control), non-diabetic with insufficient Vit D level, diabetic with sufficient Vit D level and diabetic with insufficient Vit D level. * mean significant difference at p<0.05.

In the qPCR experiment, the results showed that there was a significant decline in VDR expression in all groups in comparison to the control-group as shown in **Figure 3**.

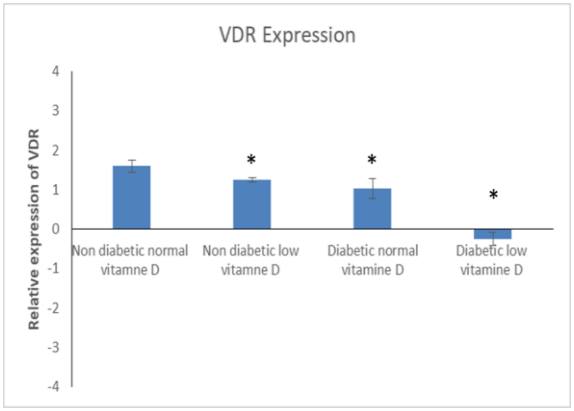

Figure 3. VDR expression in Nondiabetic with sufficient Vit D level (Control), non-diabetic with insufficient Vit D level, diabetic with sufficient Vit D level and diabetic with insufficient Vit D level. * mean significant difference at p<0.05.

T2DM represents a growing problem worldwide due to its predisposing factors such as genetics, lifestyle, environmental and nutritional factors(14). Many studies try to describe the role of Vit D levels in glucose, lipid, and calcium homeostasis(15),(16), (17). Vit D belongs to a family of lipoprotein-like hormones well known for their metabolic characteristics and their impact on many homeostasis processes. Vit D interferes with many aspects of cell biology that are regulated(18). For example, immune modulation(19) as well as cell growth and differentiation(20). It is well known that hypovitaminosis D leads to both the trigger of

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insulin resistance(21) and the eventual development of diabetes caused by cell death through excessive Ca^{+2} presence and ROS formation. Vit D significantly decreases inflammation; which is play a key role in the emergence of insulin resistance. (22).

The results of this study revealed that even in normoglycemic Vit D deficient subjects, all glycemic glycation indexes except fructosamine showed significant elevation and this may related to the initiation of insulin resistance build-up. Fructosamine level reduction in this group may related to the initiation of a pro-inflammatory process due to insulin resistance as inflammation inverts the albumin globulin ratio, reducing glycation-binding sites (albumin).

Vit D deficiency represents an important health problem in many developing countries like Iraq, especially in patients with chronic diseases due to the many biological functions that regulated to by Vit D(23).

Despite the fact that HbA1c has been considered the cornerstone of glycemic control monitoring for many years, recently there are many studies described 20-40 % variation in the HbA1c level due influenced by a variety of biological factors, including age, glucose metabolism, erythrocyte age, liver and kidney dysfunction(24). HbA1c's shortcomings have been solved by the development of Glycosylation gap, which was proposed as an alternate technique to account for glycemic fluctuations. The results of this work showed that low Vit D associated with elevation in glycosylation gap and this agree with results of many studies as Zelin et al. 2021 (25)and Faisal et al. 2018, Nagayama et al., 2020 (26) these studies described that low Vit D significantly elevated glycation processes.

This work revealed that VDR expression significantly reduced in both normoglycemic with low Vit D and the two diabetic group in compared to normoglycemic sufficient Vit D level. These results explain many of the results that obtained by other researchers as (27) Hernández-Sánchez et al., 2017and Guo et al. 2016 (28) who described the relation Vit D, VDR expression and glycosylation process.

In conclusion, VDR expression significantly reduced with elevation in glycosylation gap in both normoglycemic with low Vit D and type II diabetic with sufficient and insufficient Vit D.

Conclusions

To sum up,VDR expression significantly reduced with elevation in glycosylation gap in both normoglycemic with low Vit D and type II diabetic with sufficient and insufficient vit D group.

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Author Contributions

The study conception, design and data collection was carried out by Professor Muhammad Alkataan. Hayder Alhamdany and Alaa Altai participated in data analysis and interpretation of results. All authors contributed in the draft manuscript preparation and review the results and approved the final version of the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest or any personal circumstances or interest that may be perceived as inappropriately influencing the representation or interpretation of reported research results.

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