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RESEARCH

Uncovering the Mechanistic Role of vitamin D in Modulating Glycemic Pertained Parameters in Insulin-resistant Patients

Farqed K. Esmaeil[1], Mohammed KJ. Alnori[1]*, Mohammed A. Ajeel[1]

[1]*College of Pharmacy, University of Mosul, Mosul, Iraq. Address correspondence to: Mohammed KJ. Alnori, Email: alnorimkj@uomosul.edu.iq*

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■ Abstract

Background: Metabolic diseases have been increasingly reported as healthcare-challenging diseases, increasing health expenses and burdening the national health system. Identifying uniquely responsible mechanisms is lured around insulin and vitamin D cross-talk. The present study aimed to loom the translation of the cross-talk between vitamin D levels and insulin hyposensitivity-linked genetic pathways. **Methods:** To do so, serum samples were collected from patients with diabetes, metabolic syndrome, and obesity versus the control group with matched age, sex, and health status. Genes (NAT-2, IRS-1, and IGF-1) were quantified by polymerase chain reaction (PCR) alongside their correlative analysis with measured glycemic parameters, including fasting blood sugar (FBS), glycated haemoglobin (HbA1C), and HOMA-IR value. **Results:** Analysis of results demonstrated that vitamin D level is significantly ($p<0.05$) higher in the control group [25±5.1] compared to diabetic [16.5±4.4], metabolic syndrome [18.6±3.4], and obesity [14.1±4.8] groups, conversely, these groups have higher HOMA, FBS, and HbA1C significantly ($p<0.05$) compared to the control group. All patient groups have significantly ($p<0.05$) higher NAT-2 and IRS-1 compared to the control, while IGF-1 was significantly ($p<0.05$) elevated in the obese group. **Conclusion:** These findings provide valuable insights into uncovering the mechanisms involved in vitamin D's role linked to other plasma variables of proteins and genes including proteins (NAT-2, IRS-1, and IGF-1) pertained to insulin desensitization.

Keywords: Vitamin D, NAT-2, IRS-1, IGF-1, Insulin.

1. Introduction

The rate of metabolic diseases, including diabetes, metabolic syndrome, and obesity is hastily escalating, both nationwide and worldwide [1], with the exact cause obscure [2-4]. Researchers referred to multiple factors, including damage in pancreatic β-cell function, insulin hyposensitivity, and systemic immune response [5-9]. The deficit in insulin sensitivity is a fundamental factor, being crucial to comprehend how lifestyle modalities, like diet, exercise, and even alcohol consumption and smoking, can impact the development of type 2 DM [10, 11]. Recent studies have highlighted a potential conjoined between insulin hyposensitivity and vitamin D deficiency [12]. Vitamin D may play a key role in the pathogenesis of diabetes. Preclinical and clinical studies have shown that vitamin D can affect pancreatic β-cells and insulin hyposensitivity, steering emergent attention to its potential as a therapeutic approach for curing insulin hyposensitivity [13-15].

Latest findings have conjoined vitamin D deficiency to a broad range of health issues, including cancer, autoimmune disorders and metabolic ailments, such as type 1 and type 2 diabetes [16-18]. More than 50% of the general population is at risk for vitamin D deficiency [19]. vitamin D deficiency is marked when subjects have a serum 25-hydroxyvitamin D level lower than 50 nmol/L [20-23]. A meta-analysis of 28 studies revealed a strong connection between vitamin D levels and cardiometabolic disorders, including a 55% decrease in the risk of diabetes, a 33% decrease in the risk of cardiovascular diseases, and a 51% decrease in the risk of metabolic syndrome associated with high levels of 25-dihydroxyvitamin D [24]. The present study sought to identify the association of proteomic and genetic markers with declined vitamin D levels in diabetic, metabolic syndrome, and obese subjects.

2. Materials and Methods

Study design and settings: The study is a prospective cross-sectional study, commenced on September 2023 to March 2024, the study recruited patients from outpatient private clinics and teaching hospitals (Mosul City, Iraq). Referred patients were allocated to diabetic, metabolic, or obesity groups, after being diagnosed by a specialist endocrinologist. Those patients with thyroid diseases, cancer, pregnancy, and lactation were excluded.

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Blood collection: A 10 ml venous blood sample was collected and divided into 2 fractions, the first fraction included total blood used for instant HbA1C and genetic profiling and the second fraction of serum was separated and frozen for measuring the biochemical parameters.

RNA extraction: DNA was extracted from red blood cells using the kit supplied by AddBio (Korea) following manufacturer instructions. The RNA was quantified by Nanodrop. Primers were designed for NAT-2, IGF-1, and IRS-1 (Table 1), cDNA was generated using PCR and the Gene copy was determined for NAT-2, IGF-1, and IRS-1.

Table 1: Primers Designed.

ID gene	Sense Sequence (5' to 3')	Antisense Sequence (3' to 5')	Tm
GAPDH	ATGACATCAAGAAGGTGGTG	CATACCAGGAAAAGAGCTTG	56
IGF-1	TGGATGCTCTTCAGTTCGTG	TGGTAGATGGGGGCTGATAC	60
IRS-1	CTTCTGTCAGGTGTCCATCC	CTCTGCAGCAATGCCTGTTC	63
NAT-2	GATCACTTCCCTTGCAGACTTT	AGGCTGAATGCAATCCTCTTG	59

Statistical analysis: Analysis conducted using GraphPad Prism (Prism 8) data expressed as mean ± SD. One-way ANOVA was conducted to check for differences between groups and followed by a series of t-tests to identify the differences between each group and the other. The differences were considered significant at p value less than 0.05.

3. Results

Patients' demographic factors closely matched with control regarding age and sex with no significant differences between the groups. Regarding BMI, obesity is significantly higher than other groups, while metabolic syndrome is significantly higher than diabetic patients with control being the lowest (Table 2).

Table 2: Demographic Parameters of the Studied Group.

Groups	Control (N=18)	DM (N=20)	MS (N=20)	Obesity (N=15)
Age	49.8±4	49±4	47.9±6	48.5±3
Sex (M/F)	9/9	12/8	12/8	5/10
BMI	24.5±0.7	26.7±1	31.4±2	36.6±1.6
Duration of diseases	….	10	11	7

Analysis of results revealed that the concentration of vitamin D in diabetic [16.5±4.4], metabolic syndrome [18.6±3.4], and obesity [14.1±4.8] were significantly (P<0.05) lower than that of the control group [25±5.1], with non-significant differences existed between the patient's groups. The level of HOMA is significantly higher in diabetic [2.4±1.1], metabolic syndrome [6±4.4] and obesity [3.7±1.8] compared to the control group [2.4±1.1]. Moreover, HOMA is significantly higher in diabetic and metabolic syndrome compared to obesity. The level of FBS and HbA1C were significantly (P<0.05) higher in diabetic patients [143±18.4,6.8±1] compared to control [98.6±8.3,5.3±0.5], metabolic syndrome [110.1±17.3,5.7±0.4], and obesity [98.7±5.9,5.5±0.5], respectively (Figure 1).

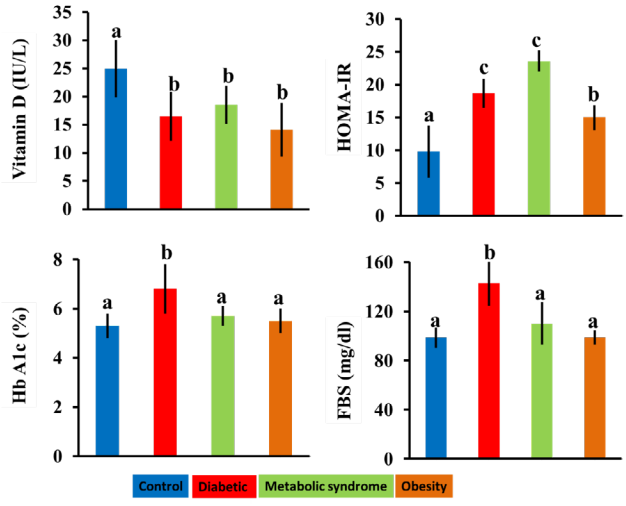

Figure 1: Vitamin D and Measured Glycemic Parameters in Control (N=18), Diabetic (N=20), Metabolic Syndrome (N=20), and Obesity (N=15) Group. Data Expressed as mean ± SD. Different Letters Express Significant Differences at p<0.05 while the Same Letters Express Non-significant Differences at p>0.05 Using One-way ANOVA to Identify Differences and Followed by a Series of Two-sample t-tests to Compare between Groups. HOMA-IR Homeostatic Model Assessment for Insulin Resistance, HbA1C= Glycated Haemoglobin, FBS=Fasting Blood Sugar.

The results of gene analysis revealed that the fold of change of gene copy of NAT-2 is higher in studied groups; diabetes [0.4] metabolic syndrome [0.8] and obesity [0.22] compared to control [0.2], with being highest in metabolic syndrome. Similarly, the results also demonstrated that the fold of change of gene copy of IRS-1 is higher in all studied groups; diabetes [59] metabolic syndrome [3482190], and obesity [23.5] compared to control [0.8], with being highest in metabolic syndrome. However, the IGF-1 fold of change of gene copy is higher in the obese group [14.2] compared to control [0.3], diabetics [0.08], and metabolic syndrome [0.05] (Figure 2).

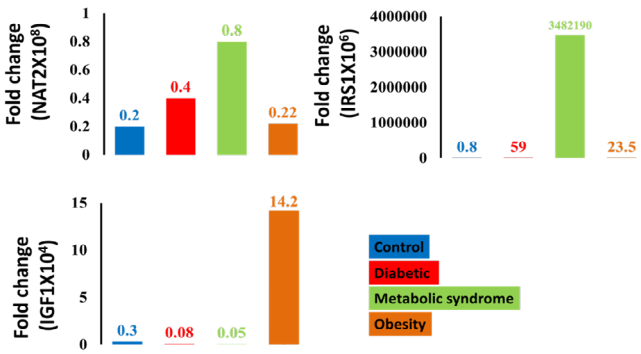

Figure 2: Gene Profiling of Fold of Changes of Measured Genes Linked to Glycemic Control and Vitamin D in Control (N=18), Diabetic (N=20), Metabolic Syndrome (N=20), and Obesity (N=15) Group. Data Expressed as Fold of Changes of Gene Copy. NAT-2= N-acetyltransferase 2, IRS-1= Insulin Receptor Substrate-1, and IGF-1= Insulin-like Growth Factor-1.

The Pearson Correlation revealed a slight positive correlation with all measured highest with IGF-1. The glycemic parameters (FBS, HBA1C, HOMA) positively associated with NAT-2, IRS-1, and IGF-1. Proteins pertained to insulin sensitivity were also positively correlated to each other (Table 3).

Table 3: Pearson Correlation of the Measured Parameters between Studied Groups.

Pearson Correlation	Vitamin D	HbAIC	FBS	HOME	NAT-2	IRS-1	IGF-1
Vitamin D	1	0.068	0.180	0.242	0.301	.20	0.79
HbAIC		1	.7950	.1500	0.054	0.112	0.179
FBS			1	.3230	.0810	0.146	0.116
HOME				1	.1640	0.064	0.087
NAT-2					1	0.035	.1460
IRS-1						1	.0210
IGF-1							1

4. Discussion

The present study has confirmed that the levels of NAT-2, IRIS1, and IGF-1 have been altered at genetic and molecular levels in metabolic derangement reciprocal to vitamin D levels. The gene expression of NAT-2 and IRS-1 elevated in diabetic and metabolic syndrome with no changes in obesity compared to the control group. IGF-1 was selectively elevated in the obesity group compared to other studied groups. These effects were translated at genetic levels in various ways, however, the pattern was the same, showing NAT-2 and IRS-1 fold of changes in the studied, which are closely related to vitamin D showing positive association.

NAT-1 and NAT-2, are cytosolic phase II enzymes responsible for the transfer of an acetyl group from Acetyl-CoA to a xenobiotic substrate. NAT-1 show wide tissue while NAT-2 is less distributed in tissues [25]. Unlike diabetes and metabolic syndrome, NAT-2 is not elevated in the obese group and is weakly related to HOMA-IR, same results achieved by Paz-Rodríguez *et al.* [26], who have reported elevated NAT-2 in diabetes and NAT-1 in obesity. Moreover, Jarrar *et al.* [27] reported an association between NAT-2 and insulin dysregulation and dyslipidemia by measuring different NAT variant alleles. NAT-2 expression is also positively expressed in metabolic syndrome patients [26]. Conversely, Knowles *et al.* [28] reported that NAT-1 (but not NAT-2) was responsible for insulin desensitization.

In vitro knockdown of NAT in cell lines has been reported to induce low synthesis of insulin-mediated glucose consumption by cells necessitating that NAT has a potential role in insulin desensitization [28], specifically NAT-2 associated with T2D, while NAT-1 is mainly modulated in obesity. In healthy subjects and diabetics, NAT-1 showed a negative conjoin with lipid profile and body fats, while this association were reported with NAT-2 in rats (Comparative to NAT-1 in humans) [29]. In NAT-1-knockout cell lines,

these changes were linked to metabolic changes in mitochondria [30], leading to derangement of lipid peroxidations explaining the low levels of NAT-1 in obese subjects. In vitro mouse adipocytes (3T3-L1 cells), NAT-2 were confirmed to be an insulin-responsive gene [28], explaining the overexpression of NAT-2 in T2D patients compared to the control group.

NAT-2 is selected because it is the most polymorphic of both enzymes [31], with a predominance of wild-type alleles in the Mexican population [32], the allelic frequency of these NAT-2 polymorphisms in T2D patients is unknown.

The correlation of NAT-2 with HbA1C and serum glucose represented in the form of that increased NAT-2 expression has a reciprocal relative impact on mitochondrial machinery and thereby reduces reactive oxygen species (ROS) [33], leading to modulation of inflammatory reaction with subsequently improved insulin sensitivity, however, the full molecular mechanism is yet to be elucidated [28].

Insulin receptor substrates (IRS-1) is a cytosol receptor that plays a role in insulin signalling [34] and is selectively elevated in metabolic syndrome in the present study. The IRS-1 play a role in binding of insulin to its receptor and hence improves glucose uptake [35] alongside playing a role in lipolysis due to the expression of IRS-1 in the fat cells [36]. Proinflammatory markers (TNFa) and ROS inhibit IRS-1 expression causing receptor desensitization [37], increasing the chance of diabetic complications due to increased blood sugar and HbA1C concentration [38]. The mechanism potentially involved the expression of SIRT1 through the synthesis of GLUT4 [39].

IGF-1 has been significantly increased selectively in obese patients compared to control or other groups at proteomic or genetic levels, moreover, a positive correlation exists highly between vitamin D and IGF-1. Obesity has been associated with hormonal changes including imbalances of thyroid hormone [40, 41] and decreased growth hormone [42-44]. Interestingly, despite decreased growth, the level of IGF-1 stayed unchanged [45-48] decreased [49, 50], or even increased in alternative studies [51-53]. This inconsistency is further compounded when looking at peak stimulated GH and IGF-1 levels, which can lead to discrepancies in identifying obese individuals with reduced GH secretion [54]. IGF-1 regulation involves an intricate complex process controlled by ghrelin, insulin, and IGF-1 binding proteins. alongside the involvement of BMI, with higher levels reported in overweight compared to normal body weight, nonetheless, severely thin or fatty individuals demonstrated the lowest level of IGF-1 [55], therefore, the connection between health conditions and IGF-1 is obscure and the need to be further elucidated [56], even conjoining IGF-1 with obesity has shown conflicting results [49, 57-59].

Unlike the present study, Soliman *et al.* [60] demonstrated that children with vitamin D deficit children exposed to a single intramuscular injection of (dose 300,000 IU vitamin D) have been associated with increased IGF-1, supported by the finding of a positive correlation between IGF-1 and vitamin D [61].

5. Conclusion

This study provides new insight into the mechanisms by which vitamin D interacts with various plasma proteins and genes, shedding light on its broader physiological roles. Specifically, research has highlighted how vitamin D may influence proteins such as NAT-2, IRS-1, and IGF-1, all of which are closely linked to insulin sensitivity and metabolic health. The interactions between vitamin D and these proteins suggest that vitamin D could play a critical role in modulating insulin desensitization pathways. Therefore, understanding how vitamin D influences these specific proteins provides a clearer picture of its potential therapeutic benefits for conditions like diabetes mellitus and metabolic syndrome. These findings underscore the importance of investigating the molecular interactions governed by vitamin D to develop more effective nutritional or pharmacological strategies for improving metabolic health outcomes.

For further studies, we recommend investigating the role of vitamin D supplementation on metabolic diseases linked to insulin-resistant diseases [diabetes, metabolic syndrome, and obesity]. Alongside, screening vitamin D in the general population and defining its role in the prediction of insulin resistance diseases [diabetes, metabolic syndrome, and obesity].

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