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ORIGINAL ARTICLE

The effect of Vitamin D on Interferon-Gamma and Biochemical Parameters in Patients with Metabolic Syndrome

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Abstract

Introduction: In this study, the effect of Vitamin D replacement on interferon-gamma (IFN-γ) cytokine and biochemical parameters has been investigated in metabolic syndrome (MetS) patients.

Methods: The research was planned as a prospective study and included 44 outpatient cases that were selected as MetS according to the IDF-2005 diagnostic criteria without overt diabetes mellitus (DM) or any inflammatory disease. The patients' Vitamin D level was <30 ng/mL, and their height, waist circumference, weight, and body mass index (BMI) measurements and biochemical parameters were recorded twice before and after 50000 IU Vitamin D replacement for 6 weeks. The IFN-γ levels were measured using the ELISA method and biochemical parameters by enzymatic colorimetric methods according to the manufacturer's instructions. The data analysis and statistical analyses were performed using IBM SPSS Statistics 22 (IBM SPSS, Türkiye) software.

Results: A statistically significant decrease was noted in the IFN-γ levels of the patients ($p<0.001$). Furthermore, statistically significant changes were noted in Vitamin D, glucose, calcium, and albumin levels, body weight, and BMI after Vitamin D replacement.

Discussion and Conclusion: The present study reveals that Vitamin D replacement leads to an anti-inflammatory effect on MetS patients as indicated by lower levels of IFN-γ and also a positive effect on hyperglycemia and BMI.

Keywords: Interferon-gamma; metabolic syndrome; vitamin D.

Metabolic syndrome (MetS) is a condition that presents with insulin resistance, subclinical inflammation, and obesity[1]. Scientific studies involving humans and animals with MetS have demonstrated the primary role of TNF-α, interferon-gamma (IFN-γ), IL-1, IL-6, IL-7, IL-8, IL-10, IL-12, IL-18, IL-21, and IL-33 cytokines in the condition[2]. Abdominal obesity is one of the leading indicators of MetS and presents with chronic inflammation[3]. Abdominal adipose tissue has been found to be associated with inflammatory processes. In healthy adipose tissue, the interleukin-10, IL-4, and IL-13 cytokines are synthesized from T-cells, eosinophils, and M2 macrophages, creating

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an anti-inflammatory environment (insulin sensitivity). In obesity, this is reversed with proinflammatory cytokines synthesized from M1 macrophages in the adipose tissue, leading to accelerated inflammation development (insulin resistance). IFN-γ is a proinflammatory cytokine that is critical for both innate and adaptive immunity, and functions as the primary activator of macrophages, in addition to stimulating natural killer cells and neutrophils[4]. Previous studies have reported IFN-γ synthesis to be inhibited when an optimal dose of Vitamin D is supplied to a lymphocyte cell culture[5]. One prospective study reported a decreased level of IFN-γ following Vitamin D replacement in patients receiving peritoneal dialysis[6]. The present study investigates the effect of Vitamin D replacement on IFN-γ cytokine and biochemical parameters in MetS patients without overt diabetes mellitus (DM) or any inflammatory disease.

Materials and Methods

The Ethics Committee Approval for the present study was granted by the University of Health Sciences, Haydarpaşa Numune Health-care Applications and Research Center Ethics Committee, with Decision No: HNEAH -KAEK 2019/41 (APPENDIX-1: Ethics Committee Approval Document). The patients, who applied to the Haydarpaşa Numune Training and Research Hospital Clinic of Internal Medicine, were informed about the study and provided their consent for inclusion. This study has been conducted according to the Declaration of Helsinki. The prospective study included 44 patients in total; of which 16 (36.3%) were male and 28 (63.6%) were female. The demographic characteristics of the patients are presented in Table 1. Patients with fatigue, excess weight gain, desire to lose weight, elevated blood pressure, and demands for referral to a dietician or for routine tests were evaluated. The study included 44 patients aged 18–70 years, who had been diagnosed with MetS according to the IDF-2005 diagnostic criteria, with Vitamin D levels of <30 ng/mL, without overt DM, and with no diseases that may cause acute or chronic inflammation. Exclusion criteria were Type 1 DM, Type 2 DM, MODA, and LADA, those undergoing Vitamin D replacement, those with a Vitamin D level of >30 ng/mL at the first admission and those who were pregnant or breastfeeding, those with hyperthyroidism, previous cerebrovascular event, chronic renal failure, chronic pulmonary disease, or nephrotic syndrome, and those with existing malignancies, autoimmune diseases, statin use, and acute inflammation (elevated CRP, fever). Waist circumference measurement was found to be high in all included patients, and hypertension (>130/85 mmHg) was measured high in 32/44 patients (72%). Regarding biochemical examination, triglyceride was tested high (>150 mg/dL) in 29/44 (66%) patients, hyperglycemia (>100 mg/dL) was found in 22/44 (50%) patients, and HDL low levels (male <40 mg/dL; female <50 mg/dL) were tested in 36/44 (81%) patients. All biochemical and IFN-γ measurements were made twice, before and after 50,000 IUs of oral Vitamin D replacement for 6 weeks in the laboratory. Human IFN-γ levels were measured with a Picokine ELISA 96 wells/kit (Boster Biological Technology, CA, Catalog number: EK0373) on an MCL-2100C ELISA device and biochemical parameters by enzymatic colorimetric methods on the Abbott Architect device according to the manufacturer's instructions.

Table 1. Demographic characteristics and anthropometric measurements

Age (years)	44.68±11.49 (22-64)
Gender (n, %)	
Female	28 (63.6%)
Male	16 (36.3%)
Height (cm)	166±17.7 (150–188)
Weight (kg)	93.15±17.7 (82–140)
Body mass index (kg/m^2)	33.56±5.88 (25.7–52.7)
Waist circumference (cm)	105±14 (82–140)
Systolic blood pressure (mmHg)	136±8.8 (119–160)
Diastolic blood pressure (mmHg)	84.9±7.3 (70–100)

Results

In this study, the average IFN-γ level was found 4.4816 (min: 0.01, max: 40.30) in the blood taken before Vitamin D replacement, while the average IFN-γ level was 1.3739 (min: 0.01, max: 3.34553) in the blood taken after Vitamin D replacement. There was a statistically significant difference in the IFN-γ levels measured before and after Vitamin D replacement ($p<0.05$). Table 2 shows the IFN-γ levels before and after Vitamin D replacement. Furthermore, a statistically significant difference has been determined in the calcium and albumin levels after Vitamin D replacement ($p<0.05$) with a negative correlation between body mass index (BMI) and Vitamin D levels ($p=0.009$; $r=-0.389$). Biochemical parameters before and after Vitamin D replacement are given in Table 3.

Vitamin D level in average was found to increase to 33 ng/mL (6.8–72) after 6-week replacement, where had been 15.39 ng/mL (4.2–29) before the replacement, which the difference was statistically significant ($p<0.05$) Furthermore, patients' average total weight was decreased from

Table 2. IFN-γ levels before and after Vitamin D replacement

IFN-γ	n	Mean	Standard Deviation	Median	Minimum	Maximum	p
Before Vitamin D replacement	44	4.4816	8.19	2.05	0.01	40.30	<0.001
After Vitamin D replacement	44	1.3739	3.34	0.01	0.01	19.76	

Table 3. Biochemical parameters before and after Vitamin D replacement

	Vitamin D replacement						p
	Before			After			
	Mean	Min.	Max.	Mean	Min.	Max.	
Glucose (mg/dL)	97.79	72.0	124.0	97.40	83.0	140.0	0.717
TG (mg/dL)	168	85	288	152	47	292	0.096
HDL (mg/dL)	42	21	60	42	25	76	0.397
LDL (mg/dL)	139	67	218	136	75	219	0.684
BUN (mg/dL)	12.5	1	20	13.3	6	19	0.179
Creatinine (mg/dL)	0.80	0.60	1.09	0.83	0.65	1.08	0.129
Uric Acid (mg/dL)	5.6	3.1	8.8	5.6	3.6	10	0.379
Magnesium (mg/dL)	1.95	1.69	2.60	1.97	1.6	3.5	0.416
Calcium (mg/dL)	9.45	8.7	10.2	9.08	8.4	10	0.000
Albumin (g/dL)	4.41	3.7	5	4.49	4	5	0.049
Leukocytes (10^3/mm^3)	7767	4700	11900	7729	4500	13000	0.687
Neutrophils (10^3/mm^3)	5976	2500	7000	4485	2300	8200	0.731
Lymphocytes (10^3/mm^3)	2518	280	4100	2430	1300	4100	0.140
Hb (g/dL)	14.08	10.3	17.8	14.18	11.4	17.5	0.450
PLT (10^3/mm^3)	270.62	111	403	271.54	172	423	0.966
ALT (IU/L)	31	10	124	25	10	112	0.061
GGT (IU/L)	34	10	95	23	10	42	0.765
MPV	9.84	7.8	12	9.93	8.1	12.4	0.165
Neutrophil/Lymphocyte ratio	2.95	0.74	33.3	1.91	0.88	3.31	0.372

93.1 kg to 91.6 kg after Vitamin D replacement, which was statistically significant (p=0.000). There was not any significant difference in systolic blood pressure (mmHg) (p=0.944) and diastolic blood pressure (mmHg) (0.297) after Vitamin D replacement.

BMI in average was measured to decrease to 33 from 33.5 (p=0.000) being statistically significant, and changes (p<0.05) were determined in the calcium and albumin levels after Vitamin D replacement. Furthermore, Vitamin D replacement had a significant positive effect on hyperglycemia (p=0.013).

As a result, the present study reveals that Vitamin D replacement leads to an anti-inflammatory effect on MetS patients as indicated by lower IFN-γ levels and also a positive effect on hyperglycemia and body mass index.

Statistics Analysis

The statistical analysis of the data was made using the SPSS version 22.0 software package. A histogram analysis and a Kolmogorov–Smirnov test were used to evaluate the normality of the distribution of variables. Descriptive statistics included mean, standard deviation, and minimum–maximum values. A Student's t-test and a Wilcoxon signed-rank test were used for normally and non-normally distributed parameters in the dependent groups. A McNemar test was used for the Chi-square test in the dependent groups. The results were evaluated at a 95% confidence interval, and p<0.05 was considered statistically significant.

Discussion

Vitamin D is believed to be associated with several inflammatory diseases; among which MetS can be counted. Yarim et al.[2] reported the inflammatory signaling pathways to

be activated and the release of proinflammatory and anti-inflammatory cytokines to be changed in MetS, resulting in biochemical and clinical disorders that can be attributed to MetS. Selimoğlu[7] found that active Vitamin D inhibited the production of IL-12 and IFN-γ, affecting the pathogenesis of inflammatory bowel disease (IBD). This is supported by the fact that exacerbations of IBD increase in winter, and IBD is more common in those living in the polar regions. Schleithoff et al.[8] determined that the serum concentration of IL-10 – an anti-inflammatory cytokine – increased and was suppressed by TNF-α-a proinflammatory cytokine – in chronic heart failure patients administered 50 µg Vitamin D supplementation per day for 9 months. A study of cell cultures by Staeva-Vieira et al.[9] reported a decrease in the IFN-γ levels under the effect of Vitamin D. Previous studies have shown the level of IFN-γ – a proinflammatory cytokine – to be high in MetS. In a prospective study by Reichel et al.,[5] it was stated that Vitamin D inhibits IFN-γ synthesis by normal human peripheral blood lymphocytes. Furthermore, Orman et al.[6] reported that the IFN-γ levels are decreased by Vitamin D replacement in patients under peritoneal dialysis.

It is understood that Vitamin D is stored in excess in adipose tissue, and Vitamin D production decreases in the skin and blood in the presence of abdominal obesity. When Vitamin D is deficient in the blood, calcium flow is provided, leading to increased adiposity, and hence, lipogenesis is stimulated in adipocytes. The present study established a statistically significant difference in the calcium and albumin levels after Vitamin D replacement ($p<0.05$). Karataş et al.[10] found Vitamin D levels to be negatively correlated with BMI and triglyceride levels, while in the present study, a negative correlation was determined between BMI and Vitamin D levels ($p=0.009$; $r=-0.389$).

It was also determined in the present study that the average weight of patients presenting after Vitamin D replacement decreased from 93.1 kg to 91.6 kg, which is a statistically significant difference ($p=0.000$), and the decrease in BMI was also found to be statistically significant ($p=0.000$). The patients had been recommended exercise, calorie restriction, and smoking cessation, and we believe that the noted weight loss may be attributed to such lifestyle changes or the anti-inflammatory effect of Vitamin D.

In conclusion, our present study reports that Vitamin D replacement inhibits the inflammatory process in MetS patients without overt diabetes by tracing the IFN-γ levels and leads to positive effects on hyperglycemia and BMI. Further studies should be designed to investigate the relationship between vitamin D and and weight loss.

Study Limitations

There are some limitations to our study. The relationships between abdominal obesity, other diagnostic criteria of MetS, and inflammation should be assessed in a larger sample group. A study design involving a comparison of individuals with and without MetS may measure the level of inflammation in MetS by comparing the IFN-γ levels before and after Vitamin D replacement. Another area of research may involve the assessment of blood samples over a longer study period involving the administration of additional Vitamin D doses to patients with a level that cannot be increased over 30 ng/mL after Vitamin D replacement. Failure to achieve the desired increase despite the administration of sufficient Vitamin D doses in some patients may be investigated.

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