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RESEARCH PAPER

Association between Vitamin D3 deficiency and Oxidative stress in Non-Communicable Diseases

Ahmed M. ZaKi[1], Jian L. Hussein[2], Nahla A.A. Abad Aljabar[3], Salih O. Haji[4]

1,3 Department of Medical Laboratory Science, College of Science, Knowledge University,Kirkuk Road, 44001 Erbil, Iraq.
[2]Department of Chemistry, College of Science, Salahaddin University-Erbil, Kurdistan Region, Iraq
[4]Department of Physics, College of Science, Salahaddin University-Erbil, Kurdistan Region, Iraq

A B S T R A C T:
A non-communicable disease (NCD) is a disease that is not transmissible directly from one person to another. NCDs in this study include most heart diseases and diabetes. Vitamin D3 (Vit. D3) deficiency is one of the most common nutritional deficiencies worldwide, and it raises the risk of the non-communicable diseases (NCDs), also the relation to increasing oxidative stress in NCDs patients. The present study is focusing on the relationship between Vit. D3 deficiency and oxidative stress in NCDs patients through determination of Vit. D3, malondialdehyde (MDA) - a biomarker of oxidative stress, lipid profile test and thyroid hormone levels in fasting samples from 120 people (controls and patients). The different groups showed highly significant differences in Vit.D3, and between the MDA levels. Also, significant differences were found in the in the level of Vit. D3, MDA and triglyceride (TG) within the groups in comparison to control subjects. However, no significant differences were found between control subjects and patients in the levels TSH, T3, T4, cholesterol, LDL, and HDL. Gender of patients also contributed to the variation and highly significant differences were observed between the female and male in the level of Vit.D3 ($P<0.0001$) and female subjects were found to be deficient of Vit.D3 when compared with control (20.71±1.471) and (34.54±1.336) respectively. On other hand, gender had no effect on the variation in MDA levels and no significant differences were present between the female and male subjects. The present study, suggests that, Vit.D3 deficiency may not play a very important role in the increased oxidative stress status as seen in NCDs patients.

KEY WORDS: Vitamin D3 deficiency, MDA, Oxidative stress, NCDs.
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1.INTRODUCTION :

Although Non-communicable diseases (NCDs) are mostly preventable via dietary choices and a healthy lifestyle, they are the leading cause of mortality and disability worldwide (Camps et al., 2014). Any development and accumulation allowed by modest, initially 'hidden' biomolecular alterations; can led to tissue damage, cellular malfunction and finally NCDs, and many of these modifications are associated with oxidative stress (Rani et al.,2016).

* **Corresponding Author:**
Jian Lateif Hussein
E-mail: jian.hussen@su.edu.krd
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Various oxidative stress-inducing variables, such as obesity, smoking and inflammation, are risk factors for NCDs. Addressing and identifying modifiable risk factors for NCDs in healthy subjects would boost long-term health and have a huge socio-economic impact on our ageing communities (Amelio,2021).
Vitamin D3 deficiency (50 nmol/l) has been proposed as a risk factor for NCDs. According to experimental evidence of Vit. D3 multi-direction effects, population-based studies indicate a high relationship between low Vit. D3 levels and the development of NCDs (Christakos et al.,2016; Schöttker et al., 2013).

Vitamin D3 is one of essential fat-soluble vitamins, which performs many functions in the human body. The main source of the Vit. D3 in the body by the action of sunlight (Hilger et al., 2016), it is formed from 7-dehydrocholesterol under the skin converted to D3 (cholecalciferol), and dietary Vit. D3 or D2 after absorption in the intestinal release to the blood circulation linked with globulin to take up vit. D3 by the liver is hydroxylated on the position 25 by the active enzyme Vit. D3 -25-hydroxylase, in the kidneys. Vitamin D3 is implicated in many subcellular genomic activities as well as biochemical and enzymatic interactions, and Vit. D3 concentration levels are vital in overcoming the inflammatory process, destroying parasites and microbes, minimizing oxidative stress when exposure to toxic agents in day-to-day routine, and controlling ageing effects (Ricca et al., 2018).

Free radicals compose from oxygen's molecules with a quantity of electrons. They can easily react with other molecules due to their odd number. As a result of easily reaction with other molecules, the free radicals can create enormous biochemical chain reactions in the body. The imbalance between antioxidants and the body's free radicals will lead to oxidative stress (Leser et al.,2019).

People's body naturally creates some free radicals as a result of processes such as exercise or inflammation. This is natural and is part of the body's sophisticated system of self-protection. anyone may also be exposed to free radicals in the environment. Ozone, certain pesticides and cleansers, cigarette smoke, and radiation are some of the sources. Due to that, free radicals quickly combine with other molecules, this is will cause a massive chain biological processes in the body. For example, lipid peroxidation it might cause by an excess of hydroxyl radical, causing damage to cell membranes and lipoproteins, which can lead to the creation of conjugated diene compounds and MDA (Breitenbach and Eckl, 2015).

However, many different methods and criteria for estimating Vit. D3 levels are available, but the accuracy and clarity by Holic are widely accepted. As per this technique, Vit. D3 deficiency is defined as a level of 25 (OH) D3 in the blood circulation that is less than or equal to 20 ng/ml (50 nmol/L), deficiency is defined as a level between 21 and 29 ng/ml, and sufficiency is defined as a level more than or equal to 30 ng/ml (Holick et al., 2005). The present study aimed to conduct an association between Vit. D3 deficiency and oxidative stress in patients with NCDs.

2. MATERIALS AND METHODS

2.1. MATERIALS

Thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were laboratory reagent grades from BDH, England. Serum fasting, cholesterol, triglyceride, HDL, LDL, Vit. D3, T3, T4, TSH tests were performed using Randox laboratory kits.

2.2 METHOD

2.2.1 Sample collection

The study included 95 hospitalized patients and out-patients who conducted NCDs (48 females and 47 males) aged between (20-70) years, collected in Rizgary and Hawler teaching hospitals in Erbil City, Kurdistan region – Iraq. The control group included 25 subjects (14 males and 11 females) healthy volunteers. The sample collection was completed over three months (September to December 2021).

A 5-ml blood sample was obtained for patient and control. The blood samples were collected in plane tubes, then at 3000 rpm centrifuged for 15 minutes. Serum MDA level was determined by (Mona et al., 1999).

The study was conducted in the Department of Chemistry - College of Science at Salahaddin University and the department of Medical Laboratory Science. Knowledge University-Kurdistan region-Iraq.

2.3 Statistical Analysis.

The assay results were analyzed using GraphPad Prism 8.0 software. Un-paired t-test for significance was performed (for normally distributed data) to compare between the normal control and patients.

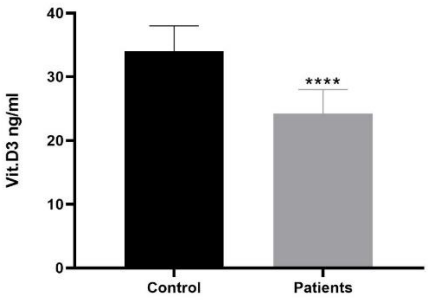
For non-parametric data(VitD3 in control group and the data of comparing VitD3 between normal and patients group), Mann-Whitney test was used to compare between the results. Data expressed as mean ± SE for parametric data and Median For non- parametric data.

3. RESULTS

Table 1; shows biostatistical and student t-test analysis (mean ± SE) for MDA parameters which were determined in this study of the total normal subjects and patients, the median value of the Vit. D 3 level in the patients is higher than control subjects (24.25 and 34) respectively. (Fig 1) showed a highly significant difference in the level Vit. D3 in the patients with NCDs with the control subjects P<0.0001, there were highly significant differences between the patients with NCDs and total control subjects in the level MDA P<0.0019 (Fig2).

Table 1. Association of serum parameters levels of the total subjects (control and patients with NCDs).

Parameters	Control subjects No. 25	Patients with NCDs No. 95	P value
Vit. D3 ng/mL (Median)	34	24.25	***P<0.0001***
MDA µmol/L (Mean ± SE)	2.756±0.1975	8.556±0.932	***P<0.0019***



.**Figure1**. Association of serum parameters levels of the total subjects (control and patients with NCDs).

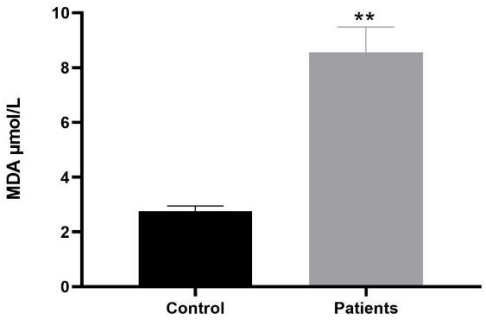


Figure 2. Association of total serum MDA (µmol/L) level for the control subjects and patients with NCDs

Regarding table 2, within the groups, a significant difference (P <0.0002) in the female groups in the level of Vit. D3 are seen when compared with control subjects (Fig3), although there was a significant difference with NCDs patients in the level of TG when compared with the control subjects at P < 0.029, (Fig4). While, there were no significant differences between patients with NCDs and control subjects in the levels TSH, T3, T4, cholesterol, LDL, HDL and MDA, respectively. In Fig (5,6) there were significant differences in the levels MDA and TG levels in male groups compared with control subjects P<0.003, P< 0.029, respectively. However, there were no significant differences in the levels of Vit.D3, TSH, T3, T4, cholesterol, LDL, and HDL

Table (3). The comparison between the male and females, (Fig 7), shows there were highly significant differences(P<0.0001) between the female and males in the level Vit.D3.

Table 2. Association of female serum parameter levels in study groups (patients with type of NCDs and controls), (Mean ± SE).

Parameters	Control subjects No. 11	Patients with NCDs No. 48	P value
Vit. D3 ng/mL	34.54±1.336	20.71±1.471	0.0002
TSH **uIU/mL**	2.234 ± 0.259	3.032 ± 0.290	0.149
T3 nmol/ml	2.331 ± 0.144	2.085 ± 0.115	0.217
T4 nmol/ml	79.47 ± 2.926	91.76 ± 5.055	0.113
Cholesterol mg/dl	159.2 ± 13.08	167.8 ± 6.514	0.598
Triglyceride mg/dl	144.7 ± 7.812	200.1 ± 11.61	0.0475
HDLmg/dl	56.63 ± 7.926	54.81 ± 2.506	0.794
LDL	75.13 ± 6.334	75.9 ± 3.543	0.932
MDA µmol/L	2.51 ± 0.2934	4.618 ± 0.4468	0.132

Table 3. Association of male serum parameter levels in study groups (patients with type of NCDs and controls), (Mean ± SE).

Parameters	Control subjects, No. 14	Patients with NCDs ,No. 47	P value
Vit.D3 ng/mL	37.43 ± 2.041	31.18 ± 1.586	0.137
TSH **uIU/mL**	3.113 ± 0.352	2.81 ± 0.294	0.554
T3 nmol/ml	1.876 ± 0.056	1.667 ± 0.123	0.185
T4 nmol/ml	78.88 ± 3.537	96.9 ± 3.649	0.100
Cholesterol mg/dl	149.1 ± 5.944	178.2 ± 5.967	0.12
Triglyceride mg/dl	155.4 ± 8.518	213.8 ± 15.9	0.029
HDL mg/dl	49.19 ± 3.671	46.76 ± 2.18	0.570
LDL mg/dl	71.31 ± 6.813	73.7 ± 4.479	0.778
MDA µmol/L	2.895 ± 0.261	9.595 ± 1.335	0.003

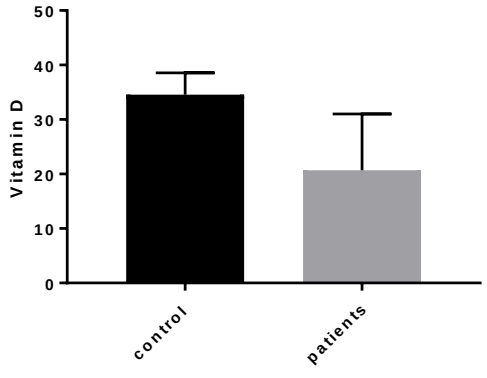


Figure 3. Association of serum Vit. D3(ng/mL) level in female control subjects and patients with NCDs

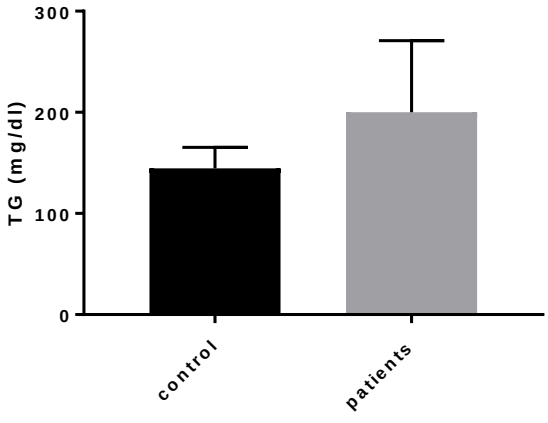


Figure 4. Association of serum TG (mg/dl) level in female control subjects and patients with NCDs

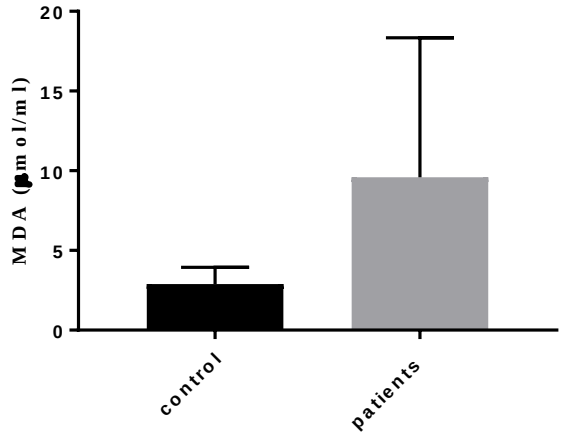


Figure 5. Association of serum MDA (µmol/L) level in male control and patient with NCDs

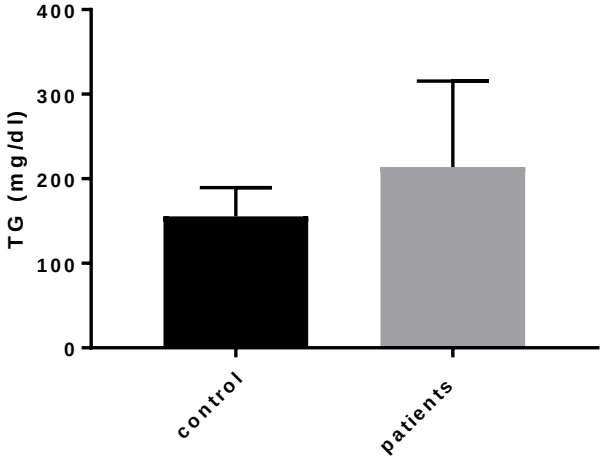


Figure 6. Association of serum TG (mg/dl) level in male control subjects and patients with NCDs

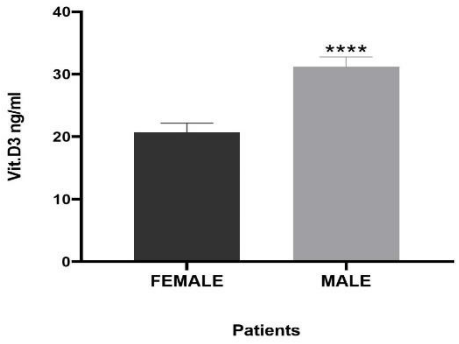


Figure 7. Association of serum Vit. D3(ng/mL) level in male and female groups patients with NCDs

4. DISCUSSION

The metabolic syndrome of stress-related is a common precursor of NCD (insulin resistance, high cholesterol, truncal obesity, and hypertension). Stress-related chronic NCDs keep haunting primary care workers, who can only halt the disease's course at most. This causes huge suffering, morbidity, and mortality, in escalating healthcare expenses (Bonadiman et al., 2017).

The current study focuses on degerming whether Vit. D3 deficiency affects lipid peroxidation and weather exposure to sunshine, diet, and gender probably affects this association. Some analyses were carried out in order to reach this goal. (Table 1) shown biostatistics and student t-test analysis mean ± SE for MDA parameter, which were determined in this study of the total normal subjects and patients, the median value of the Vit. D3 level in the patients is higher than in control subjects (24.25 and 34) respectively. This result of Vit 3 level is referred insufficient when compared with normal subjects, on the other hand, showed highly significant difference P<0.0001 with normal subjects (Fig1), also there were highly significant differences P<0.0019between the patients and total normal control in the level MDA.(Fig2). Many conducted studies suggest that there is a relation between the metabolic syndrome or its individual clinical features and the low levels of 25(OH)D3 (Gulseth et al., 2013), Vit. D3 deficiency is a cause of modest oxidative stress in the muscle, that could lead to triggering enhanced proteolysis in Vit. D3 -deficient muscles (Bhat and Ismail, 2015). This founding agreed with reported by (Câmara and Brandão, 2021).

Table2, within the groups, a significant difference (P <0.0002) in the female groups in the level of Vit. D3 are seen when comparing with normal control (Fig3). Also, there was a significant difference (P < 0.029) with NCDs patients, in the level of TG when compared with the normal subjects, (Fig4). While, there were no significant differences between patients with NCDs and normal subjects in the levels of TSH, T3, T4, cholesterol, LDL, HDL and MDA, respectively, that suggests Vit.D3 cannot modulate the oxidative stress, women with the syndrome of polycystic ovarian that administrated Vit. D3 did not significantly reduce oxidative stress (Maktabi,2018). While, there were significant differences (P<0.003) in the levels of

MDA (and TG (P< 0.029) in male groups when compared with normal subjects. Figures (5,6). However, there were no significant differences in the levels of Vit.D3, TSH, T3, T4, cholesterol, LDL, and HDL Table (3). The comparison between the males and females, (Fig 7), shows there were highly significant differences (P<0.0001) between the female and males in the level of Vit.D3, due to deficiency Vit.D3 level in the female when compare with control subjects (20.71±1.471) (34.54±1.336) respectively (Table 2); which that agreement with the reported by (AlQuaiz et al., 2018) , compared to older participants and females , teenagers and males exhibited a higher frequency of vitamin D deficiency.. On the other hand, there were no significant differences between females and males in the level of MDA. The hydroxyl radical is the most harmful to tissues because of its great reactivity and capacity to oxidize various cell components, including proteins, carbohydrates, lipids, and deoxyribonucleic acids(Frei, 1997). The lipids derivatives are produced as a result of lipid peroxidation; these differences in the results when funding in the present study, may be due to, the number of samples that have been collected it wasn't enough for both normal control and patients to record a significant result so, it cannot decide that, Vit.D3 deficiency is the cause of increased MDA level with patient's NCDs. Regarding the correlation between Vit.D3, MDA and any of the other biomarkers of interest parameters with patients' NCDs (Table 1), no significant correlation seen. The current study further highlights the difficulty in elucidating the possible relation between Vit.D3 deficiency, oxidative stress, and the risk of NCDs in a general population, due to its rare to find those with levels of 75 nmol/l or more and a few people have Vit.D3 levels above 50 nmol/l, and are extremely rare.

CONCLUSIONS

According to the findings of this investigation, Vit.D3 deficiency may not significantly impacting the increased oxidative stress status reported in NCDs patients.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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