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Relationship between *Helicobacter pylori* infections and vitamin D level and lipid profile in some obese Iraqi women

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ABSTRACT

There is a growing interest around the world for Vitamin D vital role in eradication of many infectious and non-infectious diseases, besides its effect on different biological parameters in the body, and it was the aim of this study to study the relation of Vit D levels and H.pylori infection with detection of its effect on lipid profile in presence of obesity. A total of 100 obese healthy volunteers, aged 25-60 years, were involved in this study. Their body mass index was calculated. Subjects submit to blood tests, including measurements of vitamin D (25-OH) level and lipid profile in addition to Rapid Anti H. pylori. Results revealed that there was no statistical difference in mean vitamin D level between individuals with and without *H. pylori* after age analysis and sufficient vitamin D levels significantly related to decrease in *H.pylori* infection. In *H .pylori*-positive patients vitamin D levels were lower than those in *H .pylori*-negative patients.

Key words: *Helicobacter pylori*, Vitamin D, Lipid profile, Obesity.
Article type: Research Article.

INTRODUCTION

Helicobacter pylori, one of the world's most common infections especially in the third world countries, are Gram-negative bacilli which resides the human stomach (Den Hoed & Kuipers 2020). *H. pylori* eradication is a great challenge and a matter of concerns for many scientists because treatment, and as a consequences eradication of *H. pylori*, may significantly affect the treatment of many serious *H. pylori*-associated diseases like PUD and gastric cancer (Malfertheiner *et al.* 2014). Although treatment using antibiotics is the first line of treatment strategy for *H. pylori* eradication, the problem of resistance issue always comes to the surface (Hu 2017). So, looking for the predisposing factors that may facilitate the infection with *H. pylori* could be an important approach of the treatment strategy. Vitamin D, a secosteroid, have an important role in protection against many serious diseases including cardiovascular diseases. Over 80% of metabolic vitamin D is obtained from sunlight, the remaining is acquired through dietary supplementation. There are many studies indicating the role of vitamin D deficiency as a predisposing factor for many diseases including *H. pylori* infection (Ting *et al.* 2020). Vitamin D deficiency is associated with not only *H. pylori* infection but also cardiovascular risk factors (Martini & Wood 2006; Dobnig *et al.* 2008). Insufficient vitamin D levels may cause dyslipidemia, and lipid abnormalities, an elevation in concentration of each triglyceride, total cholesterol, as well as low-density lipoprotein cholesterol concentrations and a lower concentration of high-density lipoprotein cholesterol, which was considered as a very significant predisposing factors for atherosclerosis and cardiovascular diseases (Polkowska *et al*. 2009; Potenza & Mechanick 2009). Besides, increased body mass index (BMI) has a great relationship with lower vitamin D levels (Lagunova *et al*. 2011; Earthman *et al.* 2012; Turer *et al.* 2013) clarified by the fact that obese people less exposed to sunlight, with lower cutaneous vitamin D synthesis and lower bio-

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availability of fat soluble vitamin D sequestered in the fat compartment (Wortsman *et al.* 2000). In fact, obesity itself may cause metabolic diseases, like hyperglycemia, hypertension, diabetes, and dyslipidemia. The aim of this study is to look for correlation of serum vitamin D level and lipid profile with obesity in the presence and absence of *H. pylori* infection.

MATERIALS AND METHODS

Patients

Specimens were collected under physician supervising during the period between April and December 2019 from different private clinics and hospitals in Baghdad. One hundred volunteers of Iraqi obese women, distributed as follows: group 1 (G1) include 3 women, by insufficient or normal vitamin D / *H. pylori* positive. Group 2 (G2) include 10 women by insufficient or normal vitamin D / *H. pylori* negative. Group 3 (G3) include 13 women by deficient vitamin D / *H. pylori* positive. Group 4 (G4) include 74 women by deficient vitamin D / *H. pylori* negative.

Sample Collection

A questionnaire was administered to the patients participating in the study including name, age, weight, height. Five mL of blood was collected in dry tubes without anticoagulant. After clotting, the sera were obtained by centrifugation (for 10 min at 5000 rpm) stored at -18°C until use for further examinations.

Biochemical analysis

Total serum cholesterol, triacylglycerol, LDL, HDL and VLDL levels were measured by an accredited clinical laboratory according to standard laboratory procedures. *H. pylori* antibodies Rapid Test Device (serum / plasma) was adopted as a rapid visional immune-assay for the qualitative default detection of specific Immunoglobulins M and G produced against *H. pylori* in the human sera specimens. The rapid test was done depending on manufacture instructions (ECOtest D-HP-32). The device and the specimens were left to reach room temperature, then 75 µL of serum was poured in the specimen well. Migration of specimen across the resort area in the center of the device will cause production of color (dark red color) of control band and another red band appeared within five minutes in the case of a positive result, while the only red control band appeared in the negative results. Regardless of test results, a purplish red control band in the control area should always be there. If a control band is not visible, then the test considered as invalid. Color intensity and time of color appearance were recorded for each patient.

Serum vitamin D

Serum level of vitamin D (25- hydroxyvitamin D [25(OH)D]) was measured using a radio-immunoassay RIA from the following source :(Vitamin D total, Roche Diagnostics, Mannheim, German) depending on the manufacturer's instruction. The electro-chemiluminescence binding assay was performed using Elecsys and Cobas immunoassay analyzers, with a measurement ranges of 3.00 to 70.0 ng mL^{-1} and 7.50 to 175 nmol L^{-1}. Vitamin D situation was defined depending on the classical classification as ‘‘deficient’’ (< 20 ng mL^{-1}, level 1), ‘‘insufficient’’ (20 -30 ng mL^{-1}, level 2), and ‘‘sufficient’’ (>30 ng mL^{-1}, level 3).

Statistical analysis

A package for the social science system version SPSS 20 was used for data analyzing. ANOVA-test was done to calculate previous studies. P-value equal or less than 0.05 was considered as the level of statistically significance.

RESULTS

Subject analysis

One hundred obese women were involved in the current study, with an age ranging between 25-60 years and a median age of 37 year. Patients were divided into four groups, frequency distribution of numbers and percentage of the four groups are listed in Table 1, while Fig. 1 shows the technique used to detect *H. pylori* infection (the rapid test). Table 2 and Fig. 2 illustrate the deferent lipid profile values between groups 1 and 2 in the presence of obesity factor with no observed differences in age or sex.

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Table 1. Frequency and percentage of distribution in the studied groups.

Groups	Study groups	Number (%)
G1	Insufficient vit. D / *H. pylori* positive	3(3%)
G2	Insufficient vit. D / *H. pylori* negative	10(10%)
G3	Deficient Vit. D / *H. pylori* positive	13(13%)
G4	Deficient Vit. D/ *H. pylori* negative	74(74%)

Fig. 1. *H. pylori* antibodies Rapid Test Device (positive results to the left and negative to the right).

Table 2. Comparative analyses between G1and G2 groups.

Tests	G1: NORMAL (V D3)/ H POS. NO. 3		G2: NORMAL (V D3) /H NEG. NO. 10		p-value
	Mean	**Std. Deviation**	**Mean**	**Std. Deviation**	
D3*	31.0	0.6	30.0	3.2	NS
AGE	36.7	9.2	37.4	7.9	NS
BMI*	31.5	1.6	28.5	1.9	0.05
SCHO*	231.7	19.6	211.4	28.8	0.01
STRI*	219.0	13.0	194.8	37.8	0.01
SVLDL*	42.6	2.6	43.0	11.8	NS
SHDL*	60.6	12.1	50.3	9.0	0.01
SLDL*	139.7	15.9	126.2	12.6	0.01

*NV of BM1 (Body mass index): (Less than 18.5 kg m^{-2} mean decreases in weight) (18.5-24.9 kg m^{-2} mean healthy weight) (25-30 kg m^{-2} mean obesity). *- NV of SCHO (serum cholesterol): up to 200 mg dL^{-1}. *- NV of STRI (serum triglyceride): up to 150 mg dL^{-1}. *- NV of SVLDL (Serum very low density lipoprotein): up to 35 mg dL^{-1}. * NV of SHDL (Serum high density lipoprotein): up to 50 mg dL^{-1}.*- NV of SLDL (Serum Low Density lipoprotein): up to 180 mg dL^{-1}.

In the same manner, Table 3 and Fig. 3 reveal deferent lipid profile values between groups 3 and 4 of the examined patients in the presence of obesity factor.

And to compare the parameters between the four groups of the study, the statistical analysis was done among the four groups and presented by p value equal or less than 0.05 to be considered as the level of statistically significance as shown in table 4 and the two Figs. 3 and 4.

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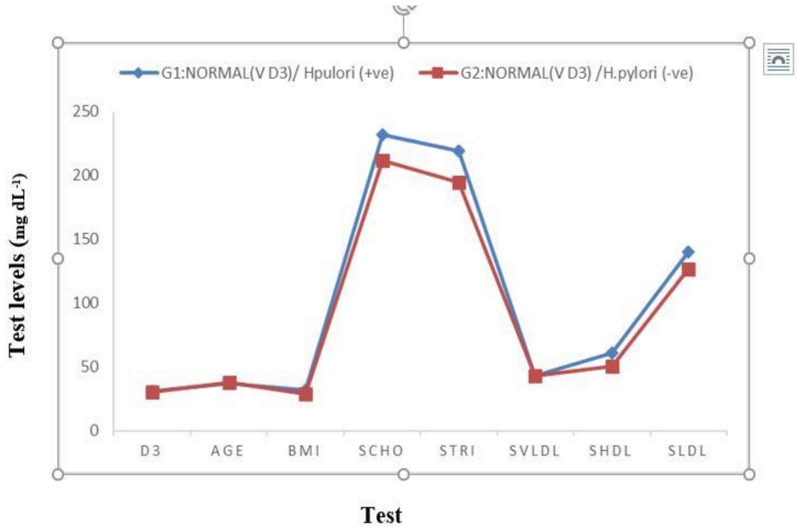

Fig. 2. Comparative analyses between G1and G2 groups.

Table 2. Comparative analyses between G3 and G4 groups

Tests	**G3:DEF (V D3) / H POS. NO. 13**		**G4:DEF (V D3) /H NEG. NO. 74**		**P value**
	Mean	**Std. Deviation**	**Mean**	**Std. Deviation**	
D3	15.2	4.4	19.7	4.3	0.01
AGE	40.7	9.8	39.4	6.9	NS
BMI	28.5	3.7	31.1	2.9	0.05
SCHO	235.7	28.3	208.4	15.6	0.01
STRI	189.3	43.1	177.3	15.2	0.01
SVLDL	37.8	8.6	35.4	7.1	NS
SHDL	51.2	5.5	46.4	6.6	0.01
SLDL	137.7	26.9	124.9	23.5	0.01

**NV of BM1 (Body mass index): (Less than 18.5 kg m^{-2} mean decreases in weight) (18.5-24.9 kg m^{-2} mean healthy weight) (25-30 kg m^{-2} mean obesity). *- NV of SCHO (serum cholesterol): up to 200 mg dL^{-1}. *- NV of STRI (serum triglyceride): up to 150 mg dL^{-1}. *- NV of SVLDL (Serum very low density lipoprotein): up to 35 mg dL^{-1}. * NV of SHDL (Serum high density lipoprotein): up to 50 mg dL^{-1}.*- NV of SLDL (Serum Low Density lipoprotein): up to 180 mg dL^{-1}.

Table 4. Comparative statistical analysis between all four tested groups.

Tests	**G1:(NO.3)*VS* G3:(N0.13)**	**G2(NO.10)*VS* G3(NO.74)**
D3	0.001	0.001
AGE	0.01	NS
BMI	0.05	0.05
SCHO	NS	NS
STRI	0.001	0.01
SVLDL	0.05	0.01
SHDL	0.01	NS
SLDL	NS	NS

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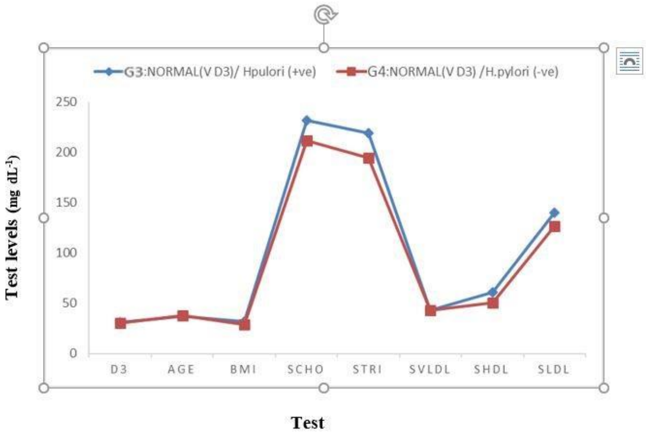

Fig. 3. Comparative analyses between G3 and G4 groups.

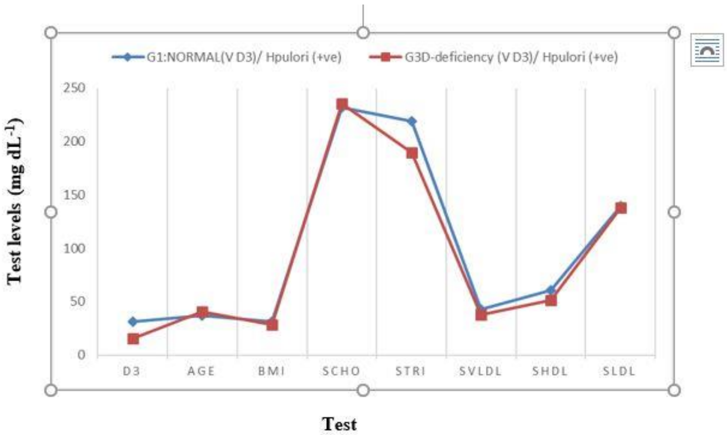

Fig. 4. Comparative statistical analysis between G1 and G3 tested groups.

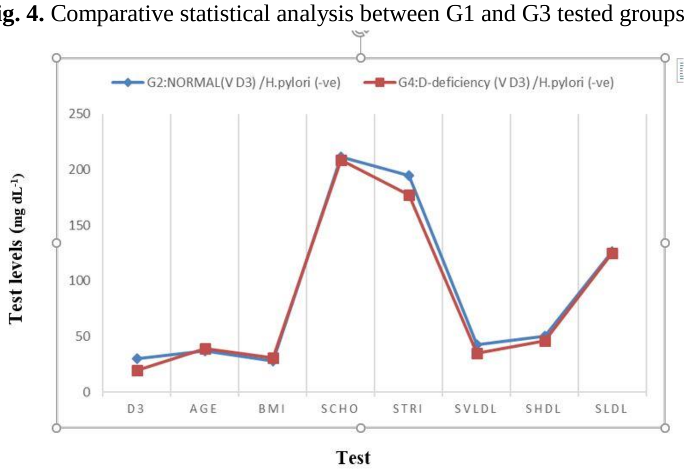

Fig. 5. Comparative statistical analysis between G2 and G4 tested groups.

DISCUSSION

Vitamin D has a role in decreasing risks of many chronic diseases, such as infectious, autoimmune and cardiovascular diseases and also cancers (Thorsen *et al.* 2017). In this study vitamin D level and *H. pylori* infection were not correlated with age, similar in line with results of Chen *et al.* which revealed that there was no statistical difference in mean vitamin D level between individuals with and without *H. pylori* after age analysis (Chen *et al.* 2016). A sufficient vitamin D concentrations were associated with decrease in *H. pylori* infection. In *H. pylori*-positive patients, vitamin D levels were lower than those in *H. pylori*-negative ones as shown also

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by Yang (2019). Lower rates of *H. pylori* eradication was associated with vitamin D deficiency (El Shahawy *et al.* 2018). Groups with vitamin D deficiency considered a risk factor related to eradication of *H. pylori*, which may lead to a need for supplementation of vitamin D before eradication of *H. pylori* (Wang *et al*. 2018). Vitamin D can reduce the risk of *H. pylori* by boosting innate immunity through up-regulating the expression of endogenous antimicrobial peptides (AMPs) including cathelicidine and defense in immune cells, revealing the vital role of vitamin D in modulating the immune response to different infectious diseases including *H. pylori* (El Shahawy *et al.* 2018). Guo *et al.* (2014) found out that in positively-infected *H. pylori* patients, the expression level of vitamin D receptor mRNA significantly and positively correlated with chronic inflammation scores in comparison with healthy control group. The results of comparing group 1 (normal D3 & *H. pylori*+) with group 2 (normal D3 & *H. pylori*-) showed a significant differences in D3 concentration, STRI, SVLDL, and BMI. Linking between *H. pylori* infection and weight gain has been hypothesized. Pilot trial was conducted in which plasma ghrelin, leptin, and gastrin levels were measured before and after *H. pylori* infection cured. These results showed that after *H. pylori* curing, plasma ghrelin (which stimulate appetite), inducing positive energy balance, and leading to gain body weight levels increased significantly by 75% ($p = 0.002$), while leptin and gastrin levels were decreased to 11% and 30%, respectively (Wanibuchi *et al*. 2018). The effect of vitamin D against *H. pylori* infection and its vital role in eradication of *H. pylori* should be determined. Vitamin D levels lowered significantly in *H. pylori* positive group than in the negative one. On the other hand, it was found that deficiency of vitamin D is significantly associated with an increased atherogenic lipids and markers of early cardiovascular disease (Ponda *et al.* 2012). Patients with vitamin D deficiency have higher serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels than those with normal levels. To evaluate the effects of vitamin D supplementation on blood lipids, A meta-analysis of randomized controlled trials of 12 clinical trials consisting of 1346 participants showed no statistically significant effects of vitamin D supplementation on TC, HDL-C and TG [differences in means were 1.52 mg dL^{-1} (-1.42 to 4.46 mg dL^{-1}), -0.14 mg dL^{-1} (-0.99 to 0.71 mg dL^{-1}) and -1.92 mg dL^{-1} (-7.72 to 3.88 mg dL^{-1}) respectively] (Wang et al., 2012). The results of the study showed dyslipidemia were higher in the vitamin D deficiency groups. In addition, for the picture to be completed, a study conducted on 2573 patients, for the period from 2008 to 2015, to detect the effect of *H. pylori* infection on serum lipid profile showed that low density lipoprotein (LDL) was higher in *H. pylori* positive patients, compared with those without the infection. Furthermore, the triglyceride (TG) concentration was higher while the level of high density lipoprotein (HDL) was lower in the cases with *H. pylori* infection, with no clear significant difference between cases with and without *H. pylori* infection (Haeri *et al*. 2018; Kazemi *et al*. 2018; Asif 2019; Madhav & Singh 2019; Arzehgar *et al*. 2019; Mitra 2020).

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