The relationship between Vitamin D status and C-reactive Protein CRP with Coronavirus Disease (COVID-19) in Iraqi Older Patients

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Abstract

Vitamin D stimulates macrophages and epithelial and immune cell development of antimicrobial peptides, which may be significant in Bacterial or viral infection eradication. Case control study was carried out from 1/August 2020 till 15 /September 2020. A total of (100) subjects from (50±2) years old age and older were attending the Health centers of Baghdad City . The study includes 100 samples divided into 2 groups (or categories) : Fifty patients with coronavirus diseases (COVID-19) and Fifty apparently healthy subjects (as control). The mean(M) ±standard error of mean (SE) of vitamin D in (ng/ml) for corona virus patients was (14.57± 1.17), while for control group was (39.70 ± 2.23),a very highly significant difference (P<0.001) was found between patients and control groups. The finding of this study shown reduced in the mean level of vitamin D less than (30 ng/ml), the mean(M) ±standard error of mean (SE) of C-reactive protein (CRP) in (ng/ml) for corona virus patients was (1.98 ± 0.15), while for control group was (0.67 ± 0.04),a very highly significant difference (P<0.001) was found between patients and control groups. All (50) patient with corona virus shown elevated in level of serum C-reactive protein (CRP) level. Weak negative correlation (-0.189) with insignificant differences (P=0.224) were observed between vitamin D and C-reactive protein (CRP).

Keywords: Vitamin D; COVID-19; immune cell; antimicrobial peptides; Serum C-reactive protein

Introduction

Vitamin D deficiency is a global public health issue. The prevalence of patients with vitamin D deficiency is highest in the elderly. Vitamin D deficiency may be related to populations who have higher skin melanin content and who use extensive skin coverage, particularly in Middle Eastern countries [1]. Vitamin D deficiency is common, affecting nearly half the US population, with higher rates among persons with darker skin or reduced sun exposure, including persons living in higher latitudes in the winter, nursing home residents, and health care workers [2].

COVID-19, caused by the SARS-CoV-2 coronavirus, often produces severe lower respiratory symptoms. Vitamin D has been identified as a potential strategy to prevent or treat COVID-19 [3]. Vitamin D treatment has been found to decrease other viral respiratory infections, especially in persons with vitamin D deficiency [4].

The aim of this study is to assess the association between vitamin D level in a sample of Iraqi corona virus patients subjects in comparison with apparently healthy subjects, and evaluate serum C-reactive protein (CRP) level in a sample of corona virus Iraqi patients as this clinical parameter has been found as an important marker that changed significantly in patients with COVID-19.

Materials and Methods

Blood sample were aspirated using disposable syringes in the sitting position. The blood is discharged slowly into plain disposable test tubes without anticoagulant. The blood was allowed to clot at room temperature for 10-15 minutes and then centrifuged at 1000xg for approximately 10-15 minutes. The sera were subdivided into two parts and stored at (-20°C) until date of analysis, in order to assess serum vitamin D and highly sensitive C-reactive protein by using ELISA technique. The kits used this study were; Human Vitamin D3 (VD3) ELISA Kit Cat No. MBS264661, Mybiosource, USA and Human high sensitivity C-reactive protein, (hs-CRP) ELISA Kit, Cat No. CSB-E08617h, CUSABIO, USA.

The data were examined by using the IBM

SPSS version 25.0, for normality, homogeneity and normal distribution, mean ± SE of mean. The probability also examined by using student T-test and ANOVA table. For non-parametric data, Pearson's chi-square test used to calculate the probability.

Results and Discussion

Clinical studies demonstrated that altered levels of some blood markers might be linked with the degree of severity and mortality of patients with COVID-19, such as Vitamin D and CRP.

The comparison between vitamin D value intervals for corona virus patients and apparently healthy subjects as control was shown in table (1); the mean (M) ±standard error of mean (SE) of vitamin D in (ng/ml) for corona virus patients was (14.57± 1.17), while for control group was (39.70 ± 2.23), a very highly significant difference (P<0.001) was found between patients and control groups (Figure 1).

Table 1: The mean(M) +standard error of mean (SE) of vitamin D in (ng/ml) for corona virus patients group and control group   in the current study.

Parameter	Group	(Mean ± SE)	P-value
Vitamin D (ng/ml)	Control (NO=50)	(39.70 ± 2.23)	2.8×10^{-15}
	Case (NO=50)	(14.57± 1.17)	

T-test: the results in table (1) shown highly significant differences between case and control in Vitamin D regarding all groups in the current work.
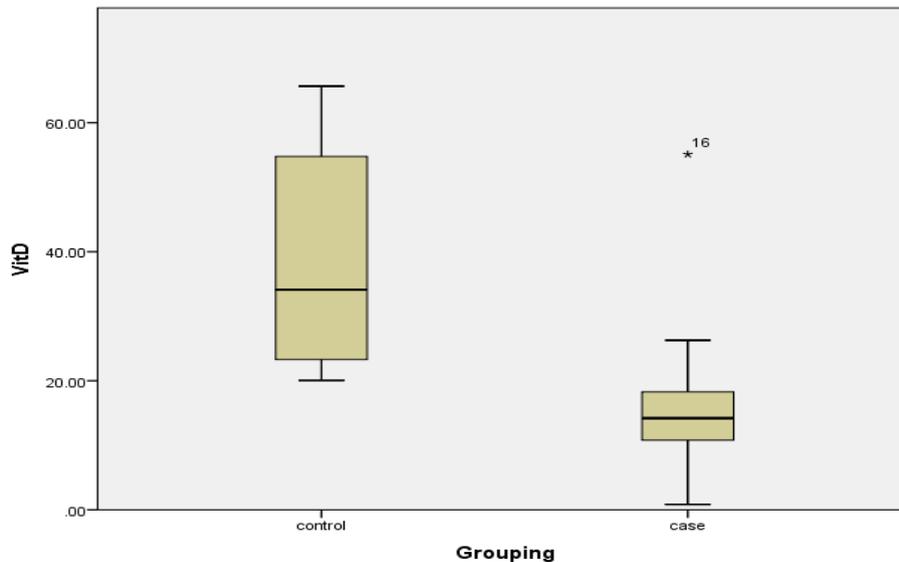
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Figure (1): Box plot of Vitamin D in (ng/ml) intervals for corona virus patients group and control group in the current study. Whiskers extending below and above the box range represent the maximum and minimum values, respectively.

In order to assess if there is any association between mean levels of vitamin D in a sample of Iraqi corona virus patients which is the first aim of this study and cases respectively mortality caused by COVID-19 ; since severe vitamin D deficiency is defined as a serum 25(OH)D lower than 30 nmol/L [5]. All (50) patient with corona virus aging patients group (≥48 years), finding of this study shown reduced in the mean level of vitamin D less than (30 ng/ml), which indicates the close link between them, this agree with Ilie *et al.* [6] who support the idea that insufficient vitamin D level increases the risk for COVID-19 and suggests that treatment for vitamin D deficiency can reduce that risk. Vitamin D status at the time of COVID-19 testing was categorized as likely deficient for patients and testing positive for

COVID-19 was associated with increasing age.

A recent meta-analysis related to vitamin D and respiratory tract infections showed that a daily or weekly Vitamin D dose between 20μg and 50μg resulted in a significant reduction of infections [7].

C-reactive protein (CRP):

In current Research, according to data analysis of C-reactive protein (CRP) in table (2): when the comparison was applied between C-reactive protein (CRP) value intervals for corona virus patients and apparently healthy subjects as control was shown in table (1); the mean(M) $\pm$standard error of mean (SE) of C-reactive protein (CRP) in (ng/ml) for corona virus patients was (1.98 ± 0.15), while for control group was (0.67 ± 0.04),a very highly significant difference (P<0.001) was found between patients and control groups.

Table 2: The mean (M) +standard error of mean (SE) of CRP in (ng/ml) for corona virus patients group and control group in the current study.

Parameter	Grouping	(Mean ± SE)	P-value
C-ractive protein (CRP) (ng/ml)	Control	(0.67 ± 0.04)	**3.5×10^{-11}**
	Case	(1.98 ± 0.15)	

T-test: the results in table 3 explain highly significant differences between case and control in CRP regarding current group in this study.
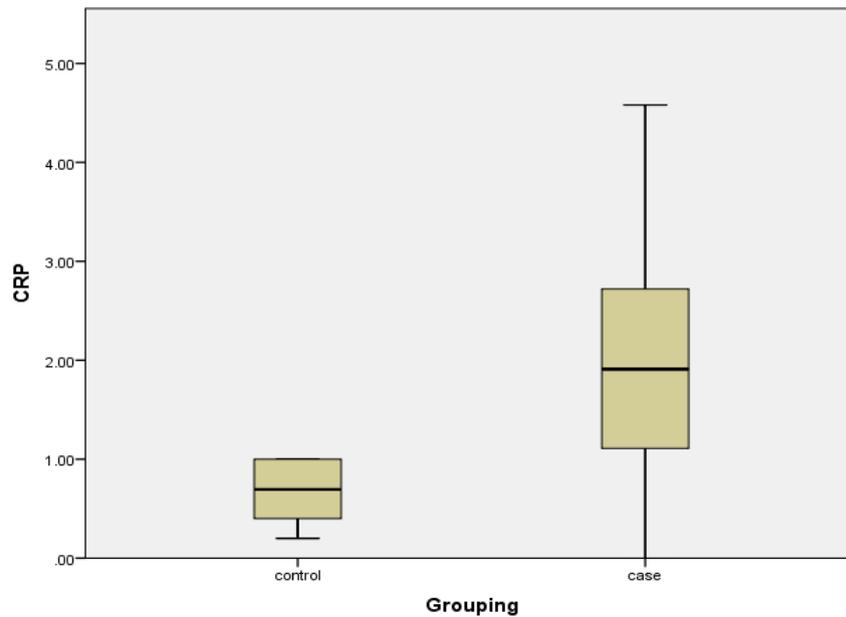
Figure 2: Box plot of C-reactive protein (CRP) in (ng/ml) intervals for corona virus patients group and control group in the current study. Whiskers extending below and above the box range represent the maximum and minimum values, respectively.

The second aim of current research is to evaluate serum C-reactive protein (CRP) level in a sample of corona virus Iraqi patients as this clinical parameter has been found as an important marker that changed significantly in patients with COVID-19. All (50) patient with corona virus shown elevated in level of serum C-reactive protein (CRP) level which agree with Ali [8]. One possible explanation for this phenomenon is the overproduction of inflammatory cytokines. Cytokines fight against the pathogen but when the system hyper activates, it can damage lung tissue.

CRP production is induced by cytokines and by tissue destruction [9].

The results of correlation coefficient (r):
Correlation relations in this research were done by applying Pearson's correlation formula.   Table (3) illustrated The Pearson's chi-square test that used to calculate the probability and the correlation coefficient between Vitamin D and CRP parameters in all study groups. Weak negative correlation (-0.189) with insignificant differences (P=0.224) were observed between this two factors according to all populations

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Table 3: Correlation coefficient of Vitamin D with CRP between Cases in the study groups.

Patients Group	(Mean ± SE)	P-value	Correlation coefficient (r)
Vitamin D(ng/ml)(cases)	(22.31 ± 1.53)	0.224	-0.189
CRP (ng/ml) (cases)	(1.5 ± 0.16)		

The last aim of this study is to investigated if any possible association was found in Iraqi corona virus patients between Vitamin D status and unregulated inflammation [10] such as C-reactive protein (CRP) which is a surrogate of cytokine storm [11]. From finding of current study; a negative correlation and non-significant difference was found that agree with Daneshkhah *et al*., [11] since this study does not prove a relationship between vit. D and CRP levels.

Conclusion

According to the study, significant relationships exist between vitamin D levels and the novel coronavirus disease COVID-19 in elderly Iraqi patients and especially the mortality caused by this infection. Vitamin D has already been shown to protect against acute respiratory infections.

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العلاقة بين مستوى فيتامين د والبروتين التفاعلي سي
مع مرض فيروس كورونا
لدى المرضى المسنين العراقيين

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الخلاصة

يحفز فيتامين د البلاعم وتطور الخلايا الظهارية والمناعية للببتيدات المضادة للميكروبات، والتي قد تكون مهمة في القضاء على العدوى البكتيرية أو الفيروسية. أجريت دراسة حالة تحكم من 1 أغسطس 2020 حتى 15 سبتمبر 2020. شارك في الدراسة ما مجموعه (100) شخص من (50 ± 2) سنة فما فوق من العمر، كانوا يترددون على المراكز الصحية في مدينة بغداد. تضمنت الدراسة 100 عينة مقسمة إلى مجموعتين (أو فئتين): خمسون مريضًا مصابًا بمرض فيروس كورونا (COVID-19)وخمسون شخصًا يبدو أنهم أصحاء (كمجموعة مراقبة). كان المتوسط ± (M) الخطأ المعياري للمتوسط (SE)لفيتامين D (بالنانوغرام/مل) لمرضى فيروس كورونا (14.57 ± 1.17)، بينما كان للمجموعة الضابطة (39.70 ± 2.23)، ووجد فرق كبير جدًا (P<0.001) بين المرضى والمجموعة الضابطة. أظهرت نتائج هذه الدراسة انخفاضًا في متوسط مستوى فيتامين د إلى أقل من (30 نانوغرام/مل)، وكان متوسط ± (M) الخطأ المعياري للمتوسط (SE) للبروتين المتفاعل (CRP) C في (نانوغرام/مل) لمرضى فيروس كورونا (1.98 ± 0.15)، بينما كان للمجموعة الضابطة (0.67 ± 0.04)، ووجد فرق كبير جدًا (P<0.001) بين المرضى ومجموعات المراقبة. أظهر جميع المرضى الخمسين المصابين بفيروس كورونا ارتفاعًا في مستوى البروتين التفاعلي (CRP) C في مصل الدم. لوحظت علاقة سلبية ضعيفة (0.189-) مع فروق غير ذات دلالة إحصائية (P=0.224) بين فيتامين D والبروتين التفاعلي.(CRP) C

الكلمات المفتاحية: فيتامين د؛COVID-19 ؛ الخلايا المناعية؛ الببتيدات المضادة للميكروبات؛ بروتين سي التفاعلي في مصل الدم